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„Dynamics of Root Exudates“

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Jeremy-James Peter BSc

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I. General Introduction

1. Root exudates

Plant roots exude a tremendous assortment of low-molecular-weight compounds into the rhizosphere (=the amount of soil that surrounds and is influenced by the roots of a plant). The complex interactions between plant roots and their surrounding environment of soil are chemical, physical and biological. Those interrelations include root-root, root-insect, and root-microbe interactions. Root exudates are regarded as a response to various biotic and abiotic stress factors (BAIS et al. 2003, 2004b).

Root exudation is based on passive and active processes. Passive root excretion includes gradient-dependent output of materials with unknown functions, and active secretion, involves exudation of compounds with known functions, such as, for example, nutrient acquisition or defence. The function of root excretions is suggested facilitating regulation of internal metabolic processes of the plant, for example respiration, while secretions facilitate external processes, such as nutrient acquisition (UREN 2000).

Multiple factors are able to affect the amounts and the composition of rhizodeposition, of which the most important are light intensity, temperature, nutritional status of the plant, activity of retrieval mechanisms, various stress factors, mechanical impedance, sorption characteristics of the growth medium, and microbial activity in the rhizosphere.

But as soon as these compounds are released in larger quantities, however, they are subjected to physical (sorption to clay minerals and humic substances), chemical (metal-catalysed oxidation), and biological (microbial degradation) processes in the soil (HUANG et al. 1999, INDERJIT & DAKSHINI 1999).

The voluminous literature on biological processes occurring in the rhizosphere suggest that each and every compound released may has a specific role or function. Although, in reality, very few suggested effects are established; some are feasible, and some, probably the majority, must remain speculative and unproven (UREN 2000).

2. The rhizosphere

The rhizosphere includes the zone up to about 2 mm distant from the root surface, the volume of soil that is believed to be influenced by the root (HILTNER 1904), and is formed around each root as it grows because the metabolic activity of each root again affects the chemical, physical, and biological properties of the soil in its proximity. Structure, particle size, water content, and buffering capacity of soil represent limiting factors to the rhizosphere. Its chemical and biological properties dramatically differ from those of the bulk soil, for example, soil acidity may increase up to10-fold in the rhizosphere (DARRAH 1993, UREN 2000).

Seventy-seven % of the total root surface is formed by root hairs. They represent the major point of contact between the plant and the soil, beyond that also providing further services for the plant, including anchorage of the plant in the soil, uptake of water and nutrients, production of substances that mediate plant-microbe associations, regulation of plant growth, and affecting microbial community structure (MICHAEL 2001).

3. Composition and chemistry of plant root exudates

The majority of root exudates comprise carbon compounds that are derived from photosynthesis, besides the ubiquitous H^+ and inorganic ions, and water. The latter are secreted in much lower quantities (UREN 2000). Table 1 provides an overview.

Table 1: Non-exhaustive enumeration of organic compounds released by plant roots (UREN 2000).

Sugars and Polysaccharides	Arabinose, fructose, galactose, glucose, maltose, mannose, mucilages of various compositions, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose
Amino acids	α-Alanine, β-alanine, γ-aminobutyric, arginine, asparagine, aspartic, citrulline, cystathionine, cysteine, cystine, deoxymugineic, 3-epihydroxymugineic, glutamine, glutamic, glycine, homoserine, isoleucine, leucine, lysine, methionine, mugineic, ornithine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, valine
Organic acids	Acetic, aconitic, ascorbic, benzoic, butyric, caffeic, citric, *p*-coumaric, ferulic, fumaric, glutaric, glycolic, glyoxilic, malic, malonic, oxalacetic, oxalic, *p*-hydroxybenzoic, propionic, succinic, syringic, tartaric, valeric, vanillic
Fatty acids	Linoleic acid, linolenic acid, oleic acid, palmitic acid, stearic acid
Sterols	Campesterol, cholesterol, sitosterol, stigmasterol
Growth Factors	*p*-Amino benzoic acid, biotin, choline, *N*-methyl nicotinic acid, niacin, pantothenic, vitamins B_1 (thiamine), B_2 (riboflavin) and B_6 (pyridoxine)
Enzymes	Amylase, invertase, peroxidase, phenolase, phosphatases, polygalacturonase, protease
Flavonoids	Flavonone
Nucleotides	Adenine, guanine, uridine/cytidine
Miscellaneous	Auxins, scopoletin, hydrocyanic acid, glucosides, unidentified ninhydrin-positive compounds, unidentified soluble proteins, reducing compounds, ethanol, glycinebetaine, inositol and myo-inositol-like compounds, Al-induced polypeptides, dihydroquinone, sorgoleone

Apical root cap and epidermal cells also release high-molecular weight polysaccharides which, together with microbial cells and clay particles, form a substance known as mucigel, whose function seems to be lubrication facilitating root movement through the soil, and enhancement of interactions between roots and soil, especially in dry soils, where contact between roots and soil is reduced.

Exudate components that potentially influence the growth and development of surrounding plants and soil micro-organisms include sugars and simple polysaccharides, amino acids, organic acids as well as phenolic compounds (HALE et al 1978, ROVIRA 1969, UREN 2000). Root metabolites usually are classified according to their deposition mechanisms (UREN & REISENAUER, 1988). A further approach to classify them can be carried out on the basis whether they have assigned a functional (excretions and secretions) or a non-functional role (diffusates and root debris). The following table presents the common definitions of root exudate classes.

Table 2: Classification of root exudates into groups regarding which functions they facilitate (CURL & TRUELOVE 1986).

Diffusates	Sugars, organic acids, amino acids, water, inorganic ions, oxygen, riboflavin, etc.
Excretions	Carbon dioxide, bicarbonate ions, protons, electrons, ethylene, etc.
Secretions	Mucilage, protons, electrons, enzymes, siderophores, allochemicals, etc.
Root debris	Root-cap cells, cell contents, etc.

Although a wide range of compounds is released, only secretions contain compounds that seem to have a direct and immediate functional role in the rhizosphere.

4. Mechanisms of root exudation

Root exudates are released in three major modes from living roots.

4.1. Diffusion

This only applies to low-molecular-weight organic compounds that are released in a passive process, in which they follow a concentration gradient between the cytoplasm of the intact root cells (millimolar range) and the soil (micromolar range). Diffusion depends on membrane permeability which further is affected by the physiological state of the root cell and the polarity of the compounds to be exuded. Accordingly, lipophilic substances are favoured by this method (GUERN et al. 1987). Also, membrane permeability can be altered by nutrient deficiency (K, P, Zn), temperature extremes (speed of diffusion and membrane permeability change), or oxidative stress (JONES & DARRAH 1995, ROVIRA 1969).

4.2. Ion channels

During nutritional deficiency, stress, or Al toxicity, exceptionally high amounts of certain organic acids, e.g. citrate, malate, or oxalate are released by ion channels (NEUMANN et al. 1999).

4.3. Vesicle transport

Higher-molecular-weight compounds are usually transported with the help of vesicles, for instance mucilage polysaccharides across the root cap with Golgi vesicles, or ectoenzymes (e.g., acid phosphatase, peroxidase) from their site of biosynthesis to their place of action through transfer vesicles (NEUMANN & ROMHELD 2000).

5. Exudation intensity

Factors that affect the intensity of root exudation include the age of the plant (decreasing), stress (increasing), such as drought and low nutrient supply, the change of light intensity (a large proportion of the organic carbon released into the rhizosphere is derived from photosynthesis; see table 3). Differences between certain families of plants, species, and cultivars were also noted (BRADY & WEIL 1999).

Table 3: Rough estimates of the fate of carbon fixed by soil-grown plants. Amounts and relative proportions depend on species, cultivars, environmental conditions, health, age, level of chemical, physical and biological stress, and so on (UREN 2000).

- Photosynthesis = 100%
  - Shoots = 50%
    - Shoots = 45%
    - Respiration = 5%
  - Roots = 50%
    - Root biomass = 25%
    - Root products = 25%
      - Respiration = 15%
      - Root debris = 10%
      - Diffusates < 1% (guess)
      - Secretions < 1% (guess) includes mucilage (and maybe more)

From the photosynthetically fixed C approximately 50% are transferred to the roots (Table 3), from which again 50% are constituted as root tissue and the other 50% are used for root products. From this 50% committed to root products again 60% (15% of the net fixed C) are lost through root respiration and just this last 40% (10%) make up root debris, secretions and diffusates. This order also reflects their hierarchy concerning exuded amounts (WHIPPS 1990).

However, there are also active retrieval mechanisms for sugars and amino acids which are capable of recovering up to 90% of the exudates passively lost into the rhizosphere, so that the carbon flow is not strictly unidirectional from root to soil. There are also reports about similar re-uptake mechanisms for nitrogen (N), and even phytosiderophores, but until now not for carboxylates. This retrieval mechanisms represent an ecological advantage due to improved nitrogen and Fe acquisition and to limitation of carbon loss (JONES & DARRAH 1993).

6. Types of root exudates and their possible functions

In root exudates, one may find every substance that is produced by the plant apart from chlorophyll and related compounds associated with photosynthesis. It is doubtful and too teleological, however, to assume that all of those have a specific function instead of representing not simply by-products of growth processes. Therefore, root products are classified into various types on the basis of their (1) chemical properties, such as composition, solubility, stability (e.g., hydrolysis, oxidation), volatility, molecular weight etc.; (2) site of origin; and (3) established, not just perceived functions.

The behaviour, biological activity and persistence of this compounds in soils is more or less determined by their chemical properties, particularly their sorption to clay minerals and humic acids, and their susceptibility to the oxidative milieu in the soil. In order to unfold its activity, it is necessary that a certain compound has to reach its target. In the rhizosphere, this implicates diffusion of a certain distance through the soil. On this journey, however, very likely the exudate component will be degraded by microbes or oxidation with subsequent polymerisation and sorption to fulvic and humic acids. Diffusates may be more mobile, but most likely suffer the same fate (GERKE et al. 1994).

Another way to classify secretions may be on the basis of their biological activity: phytohormones, ectoenzymes, phytoalexins, allochemicals, phytotoxins, amongst others (UREN 2000).

As already mentioned above, secretions are suggested to benefit the growth of the plant that produced them. After secretion, the following factors have potential to affect the function of an exudate component:

(1) Site of secretion appropriate or inappropriate
(2) Microbial assimilation/degradation
(3) Chemical alteration/degradation (e.g., oxidation)
(4) Sorption and persistence with or without activity (e.g., ectoenzymes)
(5) Diffusion and reaction with the target (e.g., complex with Al^{3+} or Fe^{3+})
(6) Mechanism of uptake (e.g., re-absorption by the root)

Table 4 summarizes the feasible functions which were allocated to specific groups of root exudate components.

Table 4: Possible functions of root exudates and involved processes (UREN 2000).

Acquisition of nutrients	
Fetchers	Seek and fetch (e.g., phytosiderophores)
Modifiers	Modification of the rhizosphere soil with e.g., protons, reductants.
Ectoenzymes	Convert unusable organic forms to usable ones (e.g., phosphatase)
Acquisition of water	Modification of the rhizosphere soil with mucilage
Protection against physical stress	Response to high soil strength through modification of interface through lubrication and amelioration of rhizosphere soil
Protection against pathogens	Defensive response to invasion (e.g., phytoalexins)
Protection against toxic elements	Response to toxic entity (e.g., complexation of Al^{3+})
Protection against competition	Modification of rhizosphere soil with phytoactive compounds (e.g., allelochemicals)
Establishment of symbiotic relationships	Chemotactic response
Rhizobia	
Endomycorrhiza	
Ectomycorrhiza	

7. Root exudate effects on nutrition

7.1. Phosphorous

As the availability of phosphorous (P), especially in its inorganic form (P_i), is limited due to its occurrence as inorganic salt component, for example as sparingly soluble calcium phosphates or iron/aluminium complexes with humic acids, it represents one of the major growth-limiting factors for plants. As a response to P deficiency, specific plant species, particularly dicotyledonous species, are able to exude an increased amount of organic acids (e.g., citrate, malate, oxalate). According to present findings, citrate and oxalate seem to be the most effective organic acids in terms of P mobilisation, due to high stability constants for complex formation with Fe, Al, and Ca ions. However, for preferably effective P_i mobilisation, carboxylic acids have to be available in rather high concentrations, which are just known from the rhizosphere of a few species, such as *Lupinus albus* L. and members of the *Proteaceae* . In lower concentrations, desorption doesn't seem to be occurring in sufficient scales, but in this case, organic acids can still inhibit the fixation of newly applied P (e.g. trough fertilisers) (DINKELAKER et al. 1997, JONES 1998). Plants that are able to secrete high amounts of organic acids as a response to P deficiency accumulate organic acids predominantly in the root tissue and, moreover, in the root zones where exudation is most intense (e.g., sub-apical root zones, proteoid roots) (NEUMANN & RÖMHELD 1999a).

Furthermore, shifts in the qualitative composition of sugars in root exudates may occur, which is attributed to increased mycorrhizal colonisation, as mycorrhiza are able to help the plant to take up Pi as well as other nutrients, like N, or even trace elements, such as copper and zinc (AZCON & OCAMPO 1984). Further root exudates that may contribute to the mobilisation of P in the soil are phenols. (DINKELAKER et al. 1997, TARAFDAR & MARSCHNER 1994).

7.2. Nitrogen and potassium

The composition of root exudates may differ depending on the form of nitrogen supplied as nitrate or ammonium, for example, increasing levels of nitrate concomitantly increase the exudation of carboxylates as well. Also, the inhibitory effect of nitrogen on the node formation by rhizobia is mainly caused by nitrate (WATERER 1992). Little is still known about the influence of potassium (K) supply on root exudation besides some studies reporting an ascending secretion of sugars, organic acids, and amino acids related to K limitation (KRAFFCZYK 1984).

7.3. Iron

Although Fe is one of the major soil constituents (MA & NOMOTO 1996), availability of iron to plants is strongly limited due to the easy precipitation of iron ions as insoluble salts, especially at pH levels favourable for plant growth. Consequently plants evolved special mechanisms for acquiring iron ions:

Strategy I

Fe(III) ion solubilisation is typically mediated by rhizosphere acidification and by reduction to Fe(II) within dicotyledonous plants and non-graminaceous monocotyledons. This is mediated by activation of the plasmalemma H^+-ATPase in combination with the accumulation of organic acids in the root tissue (ALCANTRA et al. 1991).

Strategy II

Grasses are characterised by exuding iron-mobilising metabolites as strategy for iron acquisition, so called phytosiderophores (PS). Those are highly effective chelators for Fe(III), which are furthermore stable even at soil pH levels > 7, what is an ecological advantage over strategy I plants (MURKAMI 1989).

7.4. Other micro-nutrients and heavy metals

Similarly as for iron, mobilisation of micro-nutrients such as Zn, Mn, Cu, and Co and of heavy metals (Cd, Ni) is carried out with the help of complexing agents, like organic acids or other phytosiderophores, and through rhizosphere acidification (RÖMHELD 1991).

7.5. Aluminium toxicity

In acidic soils, at soil pH levels below 5.0, aluminium (Al) toxicity is a major stress factor due to massive solubilisation of mononuclear Al species. That leads to cytotoxic effects which further on limit root growth. Again, as with P deficiency, complexation with carboxylates (citrate, oxalate, tartarate and to a lower extent also malate) seems to be the weapon of choice for plants to side-step those disadvantageous conditions, as these complexes are much less toxic and in addition can't be taken up by plant roots (KOCHIAN 1995).

The tolerance to Al toxicity is linked to the plants ability to release carboxylates in a sufficient concentration over extended periods of time, and this reaction has to occur rather fast, what denotes that this high output of carboxylates also stops as soon as Al concentration is decreasing (PELLET et al. 1995, ROVIRA 1969).

Other compounds found in root exudates and related to detoxification of Al are Al-binding polypeptides and of phosphate anions, as well as mucilage, with its high binding capacity for Al.

7.6. Conclusion from experimental evidence

Regarding all these possible functions of root exudates it has to be noted that nearly none of them actually have been observed under natural conditions. Some have been proven in hydroponic cultures, some are just assumptions according to observations under natural conditions, and a lot depend just on observations within a very narrow selection of short-lived agricultural plants. There are also a lot of problems concerning the set-up and the sampling of root exudates within an experiment. It is nearly impossible to simulate natural soil conditions, even with agricultural plants, in an experimental set-up where you also have to collect root exudates. In soils, complex reactions occur, microbial degradation, sorption, and transition metal-catalysed oxidation. As already mentioned above, there are also big concerns about the appropriate method for sample collection. Discussions continue about (1) whether it is better to do such experiments in sterile substrate (hydroponic cultures) or in soil; and (2) if a long-term experiment lasting for several weeks or days or just a short-term collection over some hours should be done. If such an experiment is executed with plants grown in soil, then again the question arises how a non-destructive collection method could be applied, because just by removing and cleaning the roots from the soil, and destroying them at least partly, you can never be sure if the substances you measure later on are exudates or happen to be just in your sample because they were released due to damage of root cells. Consequently, there exist many concerns regarding how far experimental data and effects observed under artificial experimental set-ups can be trusted and transferred from theory to practical application (NEUMANN & RÖMHELD 2000).

8. Root exudate mediated biotic interactions

Chemical signalling between plant roots and other soil organisms, including the roots of neighbouring plants, is often based on root-derived chemicals. The same chemical signals may elicit dissimilar responses from different recipients. Chemical components of root exudates may deter one organism while attracting another, or two very different organisms may be attracted with differing consequences to the plant. A concrete example of diverse meanings for a chemical signal is the secretion of isoflavones by soy-bean roots, which attract a nitrogen-fixing mutualist (*Bradyrhizobium japonicum*) and a pathogen (*Phytopthora sojae*) (MORRIS et al. 1998).

Root-root, root-microbe, and root-insect interactions may be classified as either positive or negative. Positive interactions include symbiotic associations with epiphytes and mycorrhizal fungi, and root colonisation by bacterial biocontrol agents and plant growth-promoting bacteria (PGPB). Negative interactions include competition or parasitism among plants, pathogenesis by bacteria or fungi, and invertebrate herbivory (BAIS et al. 2006).

8.1. Plant-plant interactions

Resource competition, chemical interference, and/or parasitism lead to negative interactions between plants. Root exudates have the potential to influence all three mechanisms of interference. Positive interactions between plants sometimes are also controlled by root exudates. In particular, some root exudates can induce defense responses in neighbouring plants (BAIS et al. 2006).

8.1.1. Allelopathy

In natural settings, roots are in continual communication with surrounding root systems of neighbouring plant species and quickly recognise and prevent the presence of invading roots through chemical messengers. Chemical-mediated plant-plant interference, or allelopathy, is one mechanism by which plants may gain an advantage over their competitors. Plants that produce and release potent phytotoxins can reduce the establishment, growth, or survival of susceptible plant neighbours, thus reducing competition and increasing resource availability. Plants release phytotoxins in decomposing leaf and root tissues, as green leaf volatiles, and in root exudates (BERTIN et al. 2003, WEIR et al. 2004).

Plant-produced phytotoxins vary considerably in chemical structure, mode of action, and effects on plants. Different phytotoxins in root exudates affect metabolite production, photosynthesis, respiration, membrane transport, germination, root growth, shoot growth, and cell mortality in susceptible plants (EINHELLIG 1995, WEIR et al. 2004). These effects on plant physiology, growth, and survival may in turn influence plant and soil community composition and dynamics. It also has important implications for agriculture; the effects may be beneficial, as in the case of natural weed control, or detrimental, when allochemicals produced by weeds affect the growth of crop plants.

8.1.2. Parasitic plant-host interactions

Root exudates are essential in the development of associations between parasitic plants and their plant hosts, an association that is negative for the host and positive for the parasite. More than 4000 facultative and obligate parasitic plants have been identified to date (YODER 1999). The chemical cross talk that controls the location of parasite germination and the development of physical connections between the parasite and the host is well understood for several obligate parasites, including *Striga* spp. (witch-weed) and *Orobanche* spp. (broomrape).

This host-parasite recognition is deemed to be mediated by a group of sesquiterpenes called strigolactones, after *Striga spp*. where they were firstly isolated. It seems that seeds of Striga spp., and also Orobanche, remain dormant until germination is stimulated by a chemical compound produced and exuded by the host roots. So that the parasitic weeds might find their potential hosts by detecting strigolactones, which are released from plant roots upon phosphate deficiency in communication with AM fungi (PALMER et al. 2004).

8.1.3. Induced herbivore resistance

Root exudates can also have positive effects in plant-plant interactions, although these have been less frequently reported. In particular, some root exudates increase herbivore resistance in neighbouring plants. For example, *Elytrigia repens* (couch-grass) produces several phytotoxic compounds in its root exudates, of which one, carboline, has been identified (GLINWOOD et al. 2003). *Hordeum vulgare* (barley) treated with either *E. repens* root exudates or with carboline alone was significantly less likely to be chosen as a host by aphids than control *H.vulgare* plants. Carboline in the absence of *H.vulgare* did not repel aphids, indicating that *H.vulgare* responses to *E.repens* root exudates are necessary for aphid repulsion.

8.1.4. Induced herbivore defence via predator attraction

In addition to having direct effects on herbivore behaviour, some root exudates induce defense responses in neighbouring plants that reduce herbivore populations indirectly by attracting predators and parasites of the offending herbivore (CHAMBERLAIN et al. 2001). For example, *V.faba* plants under attack release root exudates that induce green leaf volatile production in undamaged *V.faba* plants, which in turn attracts aphids parasitoids (DU et al. 1998).

8.2. Plant-microbe interactions

Specific interactions between plants and microbes mediate a wide range of essential processes in the soil such as carbon sequestration, ecosystem functioning, and nutrient cycling. As the composition of the microbial soil community significantly influences the plants ability to obtain nitrogen and other nutrients, as well as further positive and negative effects (e.g., fixation of atmospheric nitrogen, increased biotic and abiotic stress tolerance initiated by endophytic microbes, production of protective biofilms by bacteria, or negative effects through parasitic/pathogenic infection) plants on their part also try to influence this microbial associations by the dispersion of specific compounds in the rhizosphere. This rhizodeposition ranges from 10% of the net carbon rate assimilation and can rise up to more than 40% in nutrient deficient plants (BAIS et al. 2005).

8.2.1. Antimicrobial effects

As root exudates considerably increase microbial activity in the rhizosphere, rhizodeposition also enhances the likelihood of root-infection by bacteria and fungi. To prevent such infections the plant has to wage an underground war with the utilisation of phytoalexins, defense proteins, and other as yet unknown chemicals (BAIS et al. 2004a).

8.2.2. Nodulation of legumes by rhizobia

This is a very specific type of symbiosis just occurring within the Fabaceae, but not even all members of this family show associations with rhizobia, as the members of the basal subfamily *Caesalpinoideae* are mainly non-nodulating. But it is not only specific for the Fabaceae, but also very specific in its direct matter, as specific rhizobial strains just nodulate with specific host legumes. Responsible for the establishment of this relationship are signal components belonging to the class of flavonoids. Isoflavonoids are even only found in members of the legume family, and they induce nod-gene expression as well as rhizobial chemotaxis (PETERS et al. 1986).

8.2.3. Mycorrhizal associations

In contradiction to the very specific legume-rhizobia symbiosis, arbuscular mycorrhizal fungi (AMF) can be found to form associations with more than 80% of terrestrial plants. This is not an amazing fact considering that mycorrhiza increase nutrient uptake, improving plant fitness, and in turn, the associated fungi extract lipids and carbohydrates from the host root. But similar to rhizobia AMF may recognise the presence of a compatible host through root exudates, which trigger hyphal branching in the fungus That again is requisite for the built up of contact, and therefore for the establishment of symbiosis, between the two symbiotic partners. It is suggested that the production of these branching factors by the plant depends on the P-supply, with P-deficient roots secreting more active exudates.

To alleviate the penetration of the root cortex by the fungal hyphae most of the plants normal defense mechanisms are disabled (NAGAHASHI & DOUDS 1999, HARRISON 1997).

8.2.4. Plant growth-promoting bacteria (PGPB)

As already mentioned root exudates considerably increase microbial activity in the rhizosphere and some of this rhizobacteria have beneficial effects on plant growth, since they are capable to produce phytostimulators, fix atmospheric nitrogen, and secrete phytohormones such as auxins, cytokinins, and gibberellins. However, for a constant allocation of growth regulators it is mandatory that precursors of phytohormones are available in the rhizosphere (SOMERS 2004).

Another positive effect of PGPB is the establishment of “suppressive soils”, what means that they can oppress the development of soil-borne diseases caused by fungi or bacteria through competition for nutrients, niche exclusion, induced systemic resistance (ISR), and the production of antifungal metabolites (BAIS et al. 2004b).

9. Arbuscular mycorrhizal fungi

The association between plant roots and AMF, which can be found among 80% of terrestrial plants, allegorises great benefit for both partners, as the fungus improves the uptake of inorganic phosphate (Pi), but also contributes to the uptake of nitrogen (N) and various trace elements such as copper and zinc for the plant. In return obtaining carbon from his host, what is vital for him as an obligate biotroph. The plant invests up to 20% of the assimilated carbon for the formation, maintenance, and function of the AM fungal structures, an expense that is normally balanced with enhanced net photosynthesis rates (SMITH & READ 1997).

Some other advantageous effects of AMF for the plant partner are improved disease tolerance, increased drought resistance and decreased accumulation of heavy metals, but the primary benefit still seems to be improved P nutrition. This mainly happens through the extension of the extraradical mycelium, which also contains transport proteins related to Pi uptake, over distances up to several centimetres from the root (TAWARAYA 2003).

9.1. Phosphate acquisition

AMF enable plants with a limited capacity to grow at low P resources in the soil, to display normal growth rates over a broad range of external P concentrations. Normally in mycorrhized plants exist two pathways for Pi uptake, one directly at the root soil-interface, through the root epidermal cells, and the other one through the fungal mycelium. But as reported for some plant species, as soon as the association with the fungus is established, direct uptake is inactivated and the plant relies to 100% on its fungal partner for sufficient P supply. Even the high affinity Pi transporters located at the root epidermis are down regulated in response to AMF colonisation, so much the plant trusts its fungal partner (SMITH et al. 2004).

After Pi is taken up by the fungus, excess cytosolic Pi is transported into the fungal vacuoles and there condensed into polyphosphates (polyP), which further on are transported to the plant over the periarbuscular membrane into cortical cells, probably with the help of an yet unidentified anion channel, carrier, or pump (BUCHER 2007).

9.2. Nitrogen acquisition

Another nutrient whose uptake is promoted by AMF is N, which can be taken up as NH_4^+, NO_3^-, and amino acids, although the preferred constitute is NH_4^+, because for NO_3^- the fungus would have to invest extra energy to reduce it, before incorporating it into organic compounds. Furthermore AMF can increase the decomposition of organic material what also results in elevated N supply and uptake (HAWKINS et al. 2000).

In recent years the awareness of consumers about the negative influences of traditional agriculture systems on wildlife and the environment, as well as on health due to pesticide residues in food has led to an ascending demand of agricultural products derived from production not relying that strong on the use of pesticides and fertilisers, such as organic farming. As organic farming uses natural processes and cycles to supply the nutrient demand of the crops, and even to protect them from negative influences from other organisms, it is not possible until now, to reach yields as high as with conventional agriculture. Therefore it is clear that mycorrhizal fungi could attribute to a higher nutrient accommodation and further on to higher yields in organic farming, whilst reducing the demand for mineral fertilisers at the same time (ATKINSONS et. al. 2004, ATKINSON et al. 2005).

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II. Dynamics of Root Exudates

1. Introduction

Plants exude a wide variety of metabolites including sugars, sugar alcohols, amino acids, organic acids, fatty acids, sterols, and hormones, amongst others (UREN 2000, BERTIN et al. 2003, BADRI et al. 2009). Methods to assess root exudates vary. For instance, some researchers use aerated liquids media to grow the plants (e.g., BAIS et al. 2006), others solid phase microextraction in soil (e.g., WEIDENHAMER et al. 2009). The most common method is growing the plants in soil, after careful removal rinsing the roots with tap water and collect the root exudates in beakers filled with distilled water; collection times up to 24 hours are reported (SCHEFFKNECHT et al. 2006). Here we used shorter collection time of 4 hours that also is regarded as sufficient (VIERHEILIG & STEINKELLNER, personal communication). Barley (*Hordeum vulgare*), bean (*Phaseolus* vulgaris), carrot (*Daucus carota*), cucumber (*Cucumis sativa*), pea (*Pisum sativum*), pepper (*Capsicum sativum*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), and white mustard (*Sinapis alba*) were chosen to determine the broad applicability of the modified root-washing method. Reduction to 4 hours helps to keep microbial decomposition of root exudate components to a minimum. The major objective of this study is to explore if the applied method of root exudate accession in terms of sensitivity, robustness, reproducibility and comparison with literature (**Experiment 1**).

The availability of nutrients, especially that of phosphorus, is a limiting factor for plant development, but can be improved by mycorrhizal fungi that colonize the plant root (BUCHER 2007). Root exudates may contribute to the mobilization of phosphorus. Consequently, effects of root colonization by *Glomus mosae*, an arbuscular mycorrhizal fungus were compared to improved phosphorus supply to determine if root exudate patterns changes are caused by variable supply of phosphorus or colonization by mycorrhizal fungi (**Experiment 2**). Barley, carrot, cucumber, and tomato were used as model plant species.

Preliminary analyses suggested that benzoic acid represents a wide-spread stress dependent component of root exudates. The root exudates of three randomly chosen plants from the greenhouse were analysed in winter and summer to determine if seasonal differences occur in root exudation (**Experiment 3**).

2. Material and Methods

2.1. Investigated cultivars and species

2.1.1. Species variation (Experiment 1)

Nine plant species were included, all of them crop plants:

Tomato (*Solanum lycopersicum* Mill. cv. “Money-maker”, *Solanaceae*),
Potato (*Solanum tuberosum* L., *Solanaceae*),
Pepper (*Capsicum annuum* L. cv. ”WE 880 Block”, *Solanaceae*),
Pea (*Pisum sativum* L. cv. “Kleine Rheinländerin”, *Fabaceae*),
Bean (*Phaseolus vulgaris* L. cv. “Sun Gold”, *Fabaceae*),
Barley (*Hordeum vulgare* L. cv. “Montana”, *Poaceae*),
Cucumber (*Cucumis sativus* L. cv. “Hoffmanns Giganta”, *Cucurbitaceae*),
Carrot (*Daucus carota* L. cv. “Nantaise 2/Fantal”, *Apiaceae*),
White mustard (*Sinapis alba* L. cv.“Slata”, *Brassicaceae*).
For every cultivar, 3 replicates were prepared.

2.1.2. Effect of mycorrhization (Experiment 2)

Four plant species were used:
Tomato (*Solanum lycopersicum* Mill. cv. “Money-maker”, *Solanaceae*),
Barley (*Hordeum vulgare* L. cv. “Montana”, *Poaceae*),
Cucumber (*Cucumis sativus* L. cv. “Hoffmanns Giganta”, *Cucurbitaceae*),
Carrot (*Daucus carota* L. cv. “Nantaise 2/Fantal”, *Apiaceae*).
For every cultivar and every treatment, 3 repetitions were prepared.

2.1.3. Seasonal variation (Experiment 3)

Three plant species were used:
Yew tree (*Taxus baccata* L., *Taxaceae*),
Foxglove (*Digitalis purpurea* L., *Plantaginaceae*),
Begonia (*Begonia sutherlandii* L., *Begoniaceae*).
For every species and every season 3 replicates were prepared.

2.2. Mycorrhizal inoculation

The mycorrhizal fungus used for inoculation was *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG12; European Bank for the Glomales). *Glomus mosseae* belongs to the genus *Glomus,* the largest genus of arbuscular mycorrhizal (AM) fungi, containing 90 species, which all form symbiotic relationships (mycorrhizas) with plant roots. *Glomus* is the only genus in the family *Glomeraceae*, in the division *Glomeromycota*. It is highly probable that *Glomus* is a descendant of the fossil fungus *Glomites*, discovered in the Rhynie chert deposits from the Early Devonian (400 million years ago).

All *Glomus* species are obligate symbionts, as well as other AM fungi, depending on their plant host to complete their life cycle. Therefore it is not possible to culture them in the laboratory without the presence of a matching plant host. *Glomus* species are widespread and can be found in nearly all terrestrial habitats, including arable land, deserts, grasslands, tropical forests, and tundras.

Glomus species are reproducing asexually through production of spores at the tips of hyphae, either within the host root or outside the root in the soil. After those chlamydospores have germinated, the germination tube grows through the soil until it comes into contact with roots. After penetration of the root, hyphae can be formed between root cells, as well as inside root cells. For the exchange of nutrients with his host the fungus forms highly branched hyphal structures, which are coated by an invaginated cell membrane to remain within the apoplast, so called arbuscules, and food storage organs, so called vesicles, inside the root cells (BITAR-TONDO 2002).

2.3. Cultivation conditions

2.3.1. Climate chambers

Seeds were surface sterilised for 5 min in 50% commercial bleach, rinsed three times in sterile distilled water, and germinated in sterile perlite (autoclaved for 20 min at 121°C).
Inoculation of the mycorrhized plants was done as follows: Two weeks after seeding, 5 plantlets (control or inoculated) were transferred in a sterile mixture of silicate sand, expanded clay, and soil (1:1:1, by volume). All the plants were grown in a climate chamber (York International - 16 h light : 8 h dark photo-period at 23°C : 19°C light : dark; RH 50%; light intensity: 430 µE m^{-1} s^{-1}) and watered with tap water, enriched with the following nutrient solution, every other day. One L standard nutrient solution contained as follows: 472 mg $Ca(NO_3)_2$, 261 mg K_2SO_4, 136 mg KH_2PO_4, 369 mg $MgSO_4$, 8 mg NH_4NO_3, 50 mg $Fe_6H_5O_7$, 1 mg $Na_2Bo_4O_7$, 1 mg $MnSO_4$, 0.5 mg $ZnSO_4$, 0.5 mg $CuSO_4$, 0.03 mg $Al_2(SO_4)_3$, 0.03 mg $NiSO_4$, 0.03 mg $Co(NO_3)_2$, 0.03 mg TiO_2, 0.01 mg $LiCl_2$, 0.01 mg $SnCl_2$, 0.01 mg KJ; 0.01 mg KBr and 0.07 mg MoO_3 (STEINECK 1951, modified).

+P-treatments received an supply of 5ml of a P solution (KH_2PO_4/100ml H_2O) once a week. The control plants got no phosphorous nutrition at all, as well as no mycorrhiza. The mycorrhized plants also got no phosphorous nutrition at all, but therefore were mycorrhized. The plants for the comparison of exudates of different families and cultivars got just the nutrient solution described with no special treatments.

2.3.2. Plant material from greenhouse facilities

Plants were taken from greenhouse facilities at the University of Vienna, Faculty of Life Sciences, Faculty Center of Ecology. In winter (date of collection was january), *Digitalis* and *Begonia* plants were taken out of pots from the greenhouse, *Taxus* plants were also taken out of pots but from the outside garden. In summer (date of collection was august) all of the plants were taken from the outside garden, *Taxus* still out of pots, *Begonia* and *Digitalis* out of a flower bed. There was no special treatment done to any of the plants prior to collection of root exudates.

2.3.3. Experiment series

Three experiment series were performed, aiming at:

(1) Interspecific variation

(2) Mycorrhizal colonization effect

(3) Season and environmental effects

The first two series were performed in climate chambers, the third in the greenhouse (winter) and outside (summer).

2.4. Root exudates

Depending on the plant species, the inoculation period with *Glomus mosseae* ranged from 2 to 4 weeks. After the inoculation period, mycorrhizal and non-mycorrhizal plants were harvested, root exudates collected, and the degree of mycorrhization was determined (see below).

2.4.1. Root exudate collection

For each plant species and inoculation treatment, plants were harvested and the roots rinsed with tap water. To collect root exudates, the root systems were placed into beakers filled with distilled water for 4 h in the growth chamber under the same conditions as described above in the cultivation conditions section. Subsequently, the fresh mass of the roots was determined. The volume of exudate obtained was adjusted with sterile water to 20 ml per 1 g root (fresh mass). The exudates were filtered through 0.22 µm sterile filters (MicronSep™, Cellulose-nitrate, Carl Roth GmbH, Karlsruhe, Germany) and stored at –20°C until use.

2.4.2. Root exudate fractionation

Thirty ml of each exudate replicate was completely lyophilised and resolved in 2 ml of double-distilled water. Solid phase extraction was used to separate water soluble and ethanol soluble compounds from the root exudates. Amberlite XAD-1180 resin (Fluka, Sigma-Aldrich, Buchs, Switzerland) was prepared according to recommendation of the supplier. 50 ml of double-distilled water was used to elute all water soluble compounds. The same amount of ethanol was used to recover ethanol soluble compounds from the resin. Both fractions were evaporated, stored at -18 °C and resolved depending on the further analytical methods.

2.5. Mycorrhizal root colonisation

Roots were cleared (4 min in 10% KOH) and stained in a 5% ink (Shaeffer; black)/ household vinegar (= 5% acetic acid) solution according to the method of Vierheilig et al. (VIERHEILIG et al. 1998) to visualise the colonisation by *G. mossae*. The percentage of root colonisation (mean +/- SE) was determined after staining with the gridline intercept method (GIOVANNETTI & MOSSE 1980).

2.6. GC–MS sample preparation and analysis

For GC–MS measurements, dried exudates were dissolved (100µg/100µl) in *N*-methyl-*N*-TMS-trifluoroacetamide (MSTFA, Thermo Scientific) for derivatisation into trimethylsilyl ethers and esters. 0.5 µl of this solution were injected into an AutoSystem XL gas chromatograph (Perkin Elmer, Wathman MS) in the splitless mode at a temperature of 70°C, which was held for 3 minutes. A JW 5ms (18 m x 0.18 mm, 0.18 µm film thickness, Agilent Technologies Inc., Santa Barbara, CA) column was used, the helium follow rate was 0.8ml/min. The temperature gradient started at 70°C and rose to 300°C at a rate of 3°C/min. The gas chromatograph was coupled to a TurboMass quadrupol mass spectrometer (Perkin Elmer, Wathman MS). The transfer line temperature was set to 280°C, the ion source to 200°C, the filament to 70eV. The mass spectrometer was run in the TIC mode from 40 to 620 amu. Experimental time was 100 minutes.

The obtained chromatograms were integrated with Turbomas 4.1.1 and the peak areas of measured compounds where converted to relative amounts (% of the total peak area of every chromatogram). Mass spectra were tentatively identified by comparison with commercial databases (McLafferty 1997) and grouped into substance classes.

2.7. HPLC–UV sample preparation and analysis

Prior to HPLC measurements, dried exudates were diluted in methanol (Chromasolve grade) (10mg/ml). The analyses were carried out using a Dionex Summit System (Dionex, Sunnyvale, USA) equipped with a photodiode array detector and a Famos autosampler (LC Packings, Amsterdam, Netherlands). The column used was a Phenomenex Synergi Max C12 (150 x 2 mm). The particle size of the stationary phase was 5 µm. The column oven was adjusted to 40°C and the flow rate was adjusted to 0.2 ml min^{-1}. Solvent A was prepared as follows: Water (Milli-Q quality)/methanol/o-phosphoric acid (H_2O:CH_3OH:H_3PO_4 = 9:1:0.5, v/v/v), solvent B was pure methanol. The gradient started with 100% of solvent A for 2 minutes and subsequently changed linearly to 100% of solvent B within 98 minutes. Finally, this concentration was held for further 10 minutes. Five µl of each sample were injected. The UV-spectra were recorded from 220nm to 590nm and the signal wavelength was set to 229 nm. Tentative assignment of structures was carried out with Chromeleon 6.70 Build 1820 (Dionex, Sunnyvale, California, USA) and on basis of comparison with an in-house UV spectra library. The output peak areas of measured compounds where converted to relative amounts (%) of the total peak area of every sample.

2.8. Statistics

A chemometric analysis of the MS- and UV (229 nm) signals was carried out by principal component analysis (PCA). Variables, that did not contribute to grouping were excluded from the analysis. The statistical analyses were performed with the help of the following programs: SIMCA_P 11 (Umetrics, Umea, Sweden), PRIMER 6.1.8 (Primer-E Ltd., Lutton, United Kingdom) and STATGRAPHICS PLUS 5.0 (Statistical Graphics Cooperation Inc., Rockville, ML, USA).

3. Results

3.1. Interspecific variation (Experiment 1)

3.1.1. GC–MS analysis

All root exudates analysed with GC–MS yielded chromatograms with a total of 71 substances detected throughout all samples, of which 51 could be identified with the available methods (tentative characterisation according to a comparison of mass spectra and retention time). Metabolites which couldn't be identified are designated as not identified (NI) in the ongoing text. Table 1 lists all substances detected in the analysed exudate accessions.

Table 1: Substances detected with GC–MS. +, ++ and +++ = found in one, two or all 3 replicates. SL=*Solanum lycopersicum*, ST=*Solanum tuberosum*, CA=*Capsicum annuum*, CS=*Cucumis sativus*, PV=*Phaseolus vulgaris*, PS=*Pisum sativum*, HV=*Hordeum vulgare*, SA=*Sinapis alba*, DC=*Daucus carota* (for HV and PS just two accessions were available).

Compound	***Ret. time***	***SL***	***ST***	***CA***	***CS***	***PV***	***PS***	***HV***	***SA***	***DC***
Organic acids										
Oxalic acid	8.73						+	+		
Lactic acid	9.30						+	+		
Malonic acid	10.94	+++	+++	++	++	++		+	+	++
Succinic acid	15.18	+++	+++	++	+++	++	+	++	++	+++
Fumaric acid	16.66	+			++					
Malic acid	22.18	+++	+	+++	++	++	++	++	++	++
Citric acid	34.30	+		++	+				+	++
Amino acids										
Valine	11.07				+		+	++	+++	
Leucine	13.27						++	++		++
Isoleucine	13.89	+++	+++	+++	++	+++	++	++	+++	+++
Glycine	14.70		+	+	+			++	++	++
Alanine	16.46	+++	+++	+++	++	+	+	++	+++	+++
Serine	16.98			+	+			++	++	++
Threonine	17.93	+	+	++	++		++	++	++	++
Homoserine	20.52						+++		+	
Pyroglutamic acid	21.21	+++	+++	++	++			++	+++	+++
Aspartic acid	22.55				+	++		++	++	++
γ-amino-butyric acid							+		+	+
Glutamic acid	27.30							+		
Lipids components										
Glycerol	13.45				++		++			

Compound	*Ret. time*	*SL*	*ST*	*CA*	*CS*	*PV*	*PS*	*HV*	*SA*	*DC*
Glycerol-3-phosphate 1	32.28			++		+	+	+		
Glycerol-3-phosphate 2	38.40					+		+		
Glyceric acid	15.88	++			++			++	++	
Palmiticacid	41.06							+		
Sugars alcohols and acids										
Erythritol	23.47		++				++	+		
Threonic acid	24.38	+++		+	++	+		++	++	++
Ribitol	30.67							+		
Ribonic acid	31.46							++	+	++
2-keto-l-Gluconic acid	32.08					+	+	+	+	++
Glucopyranose	39.39	+		+	++		+	+	++	+
Gulonic acid	39.66	+				++		++	+++	+++
myo-Inositol	42.22	+++	+++	+++	+++	+++	++	++	+++	+++
Mannitol	43.43	+					+		+	
Galacturonic acid	49.06	+	+++	++				++	++	
Anhydrosorbitol	57.77				+					
Sugars										
Mannose	33.60				++	+	+	++		
Fructose	34.10	+++		+++	+++	+++	+	++	+++	+++
Galactose	36.50	+++		+++	++	+++		+	++	+
Glucose	36.89	+		+++	++	+	++	+	+++	+++
Cellobiose	55.79									
Sucrose	57.03	+				++			+++	++
Maltose	58.38						+			
Trehalose	59.42				++					
Melibiose	64.64	++			++					
Maltotriose	73.12				+++					++
Aromatic compounds and shikimic acid pathway derivatives										
Benzoic acid	12.17	+	+++	+++	++	+	+	++	++	++
4-Hydroxybenzoic acid	26.85									++
Benzene acetic acid	27.50			++	++			++	+++	++
Calystegin	36.88			++	+					
Quinic acid	35.16	+	+	++						++
Not identified substances (NI)										
1	9.55						++	+		
3	11.75					++		+		
4	14.41	+		++	+				++	
5	17.32					++				
6	18.05				++					

Compound	*Ret. time*	*SL*	*ST*	*CA*	*CS*	*PV*	*PS*	*HV*	*SA*	*DC*
7	19.84			+				++	++	
8	20.63								+	
9	25.50							++		+
10	28.39							+		
11	31.14				+					
12	31.35								+	
13	32.47					++				
14	33.40				+		+	++		
16	41.65									++
17	47.85			++					+	++
18	52.93								+	++
19	53.66			++	++			+	++	++
20	57.71				+				+	++
21	58.06								+	++

Analyses results for the single investigated species are listed below. The amounts are mean ± SE (% relative peak areas):

SOLANACEAE
Solanum lycopersicum (tomato)

All replicates:		
	Succinic acid	2.53 ± 0.11
	Malonic acid	0.55 ± 0.20
	Malic acid	**10.3 ± 4.1**
	Isoleucine	**18.6 ± 5.0**
	Pyroglutamic acid	**29.8 ± 7.6**
	Alanine	1.10 ± 0.36
	Threonic acid	2.37 ± 0.85
	***myo*-Inositol**	**20.0 ± 3.2**
	Galactose	0.73 ± 0.01
	Fructose	**7.46 ± 1.62**
Two replicates:		
	Glyceric acid	1.03 ± 0.02
	Melibiose	0.31 ± 0.08
One replicate:		
	Fumaric acid	0.79
	Citric acid	1.30
	Threonine	0.39
	Glucopyranose	1.77
	Gulonic acid	0.60
	Mannitol	0.93
	Galacturonic acid	0.20
	Sucrose	1.80
	Glucose	0.18
	Benzoic acid	3.63
	Quinic acid	0.38
	NI 4	4.50

Solanum tuberosum (potato)

All replicates:		
	Succinic acid	1.51 ± 0.65
	Malonic acid	0.57 ± 0.18
	Isoleucine	**50.3 ± 11.3**
	Pyroglutamic acid	**18.9 ± 6.1**
	Alanine	2.70 ± 0.62
	Galacturonic acid	0.39 ± 0.07
	***myo*-Inositol**	**5.39 ± 1.64**
	Benzoic acid	**8.18 ± 1.53**
Two replicates:		
	Erythritol	**9.93 ± 6.28**
	Quinic acid	0.78 ± 0.11
One replicate:		
	Malic acid	**12.48**
	Threonine	1.34
	Glycine	0.57

Capsicum annuum (pepper)

All replicates:		
	Malic acid	**6.98 ± 2.33**
	Isoleucine	**12.6 ± 4.3**
	Alanine	3.64 ± 0.33
	myo-Inositol	4.60 ± 0.82
	Galactose	5.41 ± 3.99
	Fructose	**27.9 ± 14.4**
	Glucose	0.93 ± 0.49
	Benzoic acid	**5.28 ± 1.16**
Two replicates:		
	Malonic acid	1.51 ± 0.17
	Succinic acid	1.41 ± 0.11
	Citric acid	1.18 ± 0.34
	Pyroglutamic acid	**36.8 ± 1.3**
	Threonine	0.80 ± 0.34
	Glycerol-3-phosphate	0.40 ± 0.04
	Galacturonic acid	0.17 ± 0.02
	Phenylacetic acid	0.61 ± 0.13
	Quinic acid	0.91 ± 0.27
	Calystegin	0.35 ± 0.03
	NI 4	2.20 ± 0.23
	NI 21	0.35 ± 0.01
	NI 23	0.61 ± 0.18
One replicate:		
	Serine	0.77
	Glycine	0.59
	Glucopyranose	0.38
	Threonic acid	0.46
	NI 7	1.01

CUCURBITACEAE
Cucumis sativus (cucumber)

All replicates:		
	Succinic acid	2.99 ± 0.52
	Myo-inositol	**8.82 ± 3.42**
	Fructose	**27.3 ± 14.9**
	Maltotriose	2.02 ± 0.72
Two replicates:		
	Malonic acid	0.55 ± 0.01

	Fumaric acid	0.65 ± 0.15
	Malic acid	**12.7 ± 1.2**
	Isoleucine	**11.6 ± 6.9**
	Threonine	0.88 ± 0.39
	Pyroglutamic acid	**26.7 ± 2.3**
	Alanine	2.21 ± 0.81
	Glycerol	**6.24 ± 2.11**
	Glyceric acid	0.84 ± 0.33
	Threonic acid	0.73 ± 0.00
	Glucopyranose	0.85 ± 0.34
	Galactose	**9.65 ± 6.16**
	Glucose	2.64 ± 1.17
	Mannose	0.58 ± 0.19
	Melibiose	0.90 ± 0.28
	Trehalose	1.12 ± 0.33
	Benzoic acid	1.02 ± 0.23
	Phenylacetic acid	0.61 ± 0.11
	NI 6	0.42 ± 0.04
	NI 23	0.57 ± 0.21
One replicate:		
	Citric acid	0.55
	Serine	2.67
	Glycine	2.60
	Valine	0.57
	Aspartic acid	0.30
	Anhydrosorbitol	0.63
	Calystegin	2.32
	NI 4	1.73
	NI 13	0.29
	NI 16	1.18
	NI 25	0.48

FABACEAE
Phaseolus vulgaris (bean)

All replicates:		
	Isoleucine	**33.1 ± 13.4**
	myo-Inositol	3.51 ± 1.55
	Galactose	4.47 ± 1.73
	Fructose	**25.1 ± 7.3**
Two replicates:		
	Succinic acid	0.51 ± 0.11
	Malonic acid	2.69 ± 1.10
	Malic acid	1.00 ± 0.28
	Aspartic acid	**8.73 ± 1.50**
	Gulonic acid	**5.13 ± 0.29**
	Glycerol-3-phosphate 2	1.75 ± 0.48
	Sucrose	2.13 ± 1.24
	NI 3	2.39 ± 0.25
	NI 5	1.75 ± 0.39
One replicate:		
	Alanine	4.64
	Threonic acid	2.30
	Glycerol-3-phosphate	0.60
	2-keto-l-gluconic acid	3.03
	Glucose	1.42
	Mannose	0.44
	Benzoic acid	**34.5**
	NI 18	0.90

***Pisum sativum* (pea)**

All replicates:		
	Homoserine	**34.5 ± 3.5**
Two replicates:		
	Malic acid	1.79 ± 0.23
	myo-Inositol	3.38 ± 0.26
	Erythritol	**29.5 ± 3.8**
	Glucose	2.95 ± 1.29
	NI 1	3.45 ± 1.95
	Leucine	3.28 ± 1.53
	Isoleucine	3.88 ± 0.27
	Threonine	4.87 ± 0.83
	Valine	3.01 ± 1.76
	Glycerol	3.44 ± 1.81
One replicate:		
	Oxalic acid	3.67
	Lactic acid	**6.98**
	Succinic acid	**17.9**
	γ-amino-butyric acid	1.33
	Alanine	**7.04**
	2-Keto-l-gluconic acid	**5.41**
	Glucopyranose	1.45
	Mannitol	0.28
	Fructose	**19.9**
	Mannose	4.42
	Maltose	1.04
	Galactose	4.64
	Benzoic acid	1.45
	NI 16	1.52

POACEAE
***Hordeum vulgare* (barley)**

All replicates:		
	Succinic acid	1.15 ± 0.37
	Malic acid	3.67 ± 1.56
	Ribonic acid	0.30 ± 0.08
	Serine	0.67 ± 0.14
	Leucine	**7.87 ± 0.52**
	Isoleucine	**15.5 ± 1.3**
	Threonine	1.88 ± 0.46
	Glycine	0.78 ± 0.06
	Valine	1.58 ± 0.18
	Pyroglutamic acid	**19.5 ± 2.8**
	Aspartic acid	1.53 ± 0.24
	Alanine	3.15 ± 0.38
	Glyceric acid	1.58 ± 0.84
	Threonic acid	1.90 ± 0.91
	Gulonic acid	0.34 ± 0.01
	Myo-inositol	0.89 ± 0.44
	Galacturonic acid	0.53 ± 0.23
	Mannose	1.81 ± 0.98
	Fructose	**12.9 ± 7.2**
	Benzoic acid	1.69 ± 0.72
	Phenylacetic acid	0.67 ± 0.01

	NI 7	2.37 ± 0.76
	NI 9	3.95 ± 2.55
	NI 16	0.33 ± 0.08
One replicate:		
	Oxalic acid	1.48
	Lactic acid	0.82
	Malonic acid	0.52
	Glutamic acid	**7.21**
	Palmitic acid	0.14
	Erythritol	**12.3**
	Ribitol	0.32
	2-Keto-l-gluconic acid	0.61
	Glycerol-3-phosphate	0.56
	Glucopyranose	0.14
	Galactose	0.16
	Glucose	0.61
	NI 1	0.16
	NI 3	0.90
	NI 10	0.25
	NI 18	0.25
	NI 23	0.37

BRASSICACEAE
Sinapis alba (white mustard)

All replicates:		
	Isoleucine	**27.0 ± 4.7**
	Valine	1.17 ± 0.27
	Pyroglutamic acid	**19.5 ± 5.3**
	Alanine	2.53 ± 0.54
	myo-Inositol	1.40 ± 0.41
	Gulonic acid	3.48 ± 0.47
	Glucose	1.14 ± 0.65
	Fructose	**13.9 ± 7.0**
	Sucrose	0.78 ± 0.26
	Phenylacetic acid	0.45 ± 0.10
Two replicates:		
	Succinic acid	0.22 ± 0.01
	Malic acid	4.24 ± 1.14
	Serine	3.94 ± 0.84
	Threonine	2.90 ± 0.43
	Glycine	0.32 ± 0.05
	Aspartic acid	**5.24 ± 3.30**
	Glyceric acid	0.32 ± 0.05
	Threonic acid	0.74 ± 0.17
	Glucopyranose	0.12 ± 0.01
	Galacturonic acid	0.61 ± 0.01
	Galactose	**9.13 ± 5.08**
	Benzoic acid	3.91 ± 0.22
	NI 4	3.74 ± 0.55
	NI 7	1.53 ± 0.09
	NI 23	0.47 ± 0.09
One replicate:		
	Malonic acid	0.15
	Ribonic acid	0.14
	Citric acid	0.88
	γ-amino-butyric acid	1.28
	Homoserine	0.73
	2-Keto-l-gluconic acid	0.48
	Mannitol	1.99
	NI 8	2.31

NI 14	1.20
NI 21	0.23
NI 22	0.31
NI 25	0.74
NI 26	0.27

APIACEAE
***Daucus carota* (carrot)**

All replicates:		
	Succinic acid	1.16 ± 0.38
	Isoleucine	**18.1 ± 3.7**
	Pyroglutamic acid	**22.7 ± 3.9**
	Alanine	1.94 ± 0.16
	Aspartic acid	**8.07 ± 4.53**
	Gulonic acid	**8.54 ± 2.28**
	myo-Inositol	0.25 ± 0.05
	Glucose	1.59 ± 0.31
	Fructose	**13.9 ± 8.6**
Two replicates:		
	Malonic acid	0.22 ± 0.07
	Malic acid	**5.31 ± 2.13**
	Ribonic acid	0.18 ± 0.05
	Citric acid	3.04 ± 0.63
	Leucine	1.27 ± 0.18
	Serine	0.45 ± 0.13
	Threonine	0.30 ± 0.10
	Glycine	0.68 ± 0.17
	Threonic acid	0.21 ± 0.03
	2-keto-l-gluconic acid	0.17 ± 0.03
	Maltotriose	0.41 ± 0.18
	4-Hydroxybenzoic acid	0.23 ± 0.01
	Benzoic acid	**6.71 ± 0.13**
	Quinic acid	0.29 ± 0.01
	Phenylacetic acid	0.78 ± 0.06
	NI 20	3.51 ± 1.90
	NI 21	0.77 ± 0.17
	NI 22	0.41 ± 0.05
	NI 23	1.72 ± 0.02
	NI 25	0.88 ± 0.37
	NI 26	0.64 ± 0.19
One replicate:		
	γ-amino-butyric acid	1.97
	Glucopyranose	0.44
	Sucrose	0.35
	Galactose	**11.5**
	NI 9	0.33

3.1.2. HPLC–UV analysis

HPLC analyses detected a total of 140 substances throughout all samples, of which 15 could be identified on basis tentative characterisation according to a comparison of UV spectra and retention time. Metabolites which couldn't be identified were designated as NI in the following. Among the identified compounds, one was allocated to the cinnamic acids, three to the amino acids, two to flavanoids, one to isoflavonoids, and 8 to aromatic compounds in general. Table 2 lists all substances detected in the analysed exudate accessions.

Table 2: Substances detected with HPLC. +, ++ and +++ = found in one, two or all 3 replicates. SL=*Solanum lycopersicum*, ST=*Solanum tuberosum*, CA=*Capsicum annuum*, CS=*Cucumis sativus*, PV=*Phaseolus vulgaris*, PS=*Pisum sativum*, HV=*Hordeum vulgare*, SA=*Sinapis alba*, DC=*Daucus carota*.

Compound	*Ret. time*	*SL*	*ST*	*CA*	*CS*	*PV*	*PS*	*HV*	*SA*	*DC*
Amino acids										
Tyrosine	4.91				++			+++	+++	
Phenylalanine	7.66							+	++	
tryptophane	14.28	+++	+++	+++	+++	++	+++	+++	+++	+++
Aromatic acids										
Vanillic acid	18.22	+			+++					+
Phenylacetic acid	20.03								++	+++
p-Coumaric acid	27.48					+		+		
Benzoic acid	30.11	+++	+++	+++	+++	++	+++	+++	+++	+++
Ferulic acid	30.72	+								++
Salicylic acid	33.65	++								
Sinapinic acid	35.70							++		
Chlorogenic acid	37.03									+
Cinnamic acid	42.89	+			+++					+
Flavanoids										
Kaempferol derivative 1	29.09								++	
Kaempferol derivative 2	42.06								+++	
Isoflavones										
Pisatin	54.13						++			
Not identified substances (NI)										
1	2.67	+++		+++	+++	+++	+		+++	+++
2	2.84	+						+++		
3	3.15			+	+				+	
4	3.86					++	++			++
5	4.34							+++	+++	
7	5.82									+
8	7.90	++			+	+		++		
9	8.64							+	++	+
10	8.79				++				++	
11	10.74	+		+	++				+++	
12	11.59							+++	+	
13	13.31								+	
14	14.84							+		
15	16.19								++	
16	16.35							+		+
18	16.77								+	

Compound	***Ret. time***	***SL***	***ST***	***CA***	***CS***	***PV***	***PS***	***HV***	***SA***	***DC***
19	17.02							+++	++	
20	18.03						+			
22	18.20							++	+++	
24	19.42								+++	
26	20.68							++		
27	20.91							++	+++	
28	21.29				+					
30	22.22			+						
31	22.60							+		
32	22.63								++	
34	23.18	+			+					
36	23.32									+++
37	23.42					++	++			
38	23.80							+++	+	
40	23.98			++	++		++			+
42	24.61								+++	
44	24.95								+	+++
45	25.03				++					
46	25.96							+++	+++	
48	26.15	++			+					+
49	26.19								+++	
52	27.50			++	+		+++		++	++
55	27.94							+		
56	28.53				++					
57	28.54								++	
58	28.58					+		+		
59	29.11									++
60	29.47				+					
62	30.83			++						
64	31.06						++			
66	31.44								+++	
68	32.23				+++					++
69	32.41			++				+		
71	33.39	+	+		+					
72	33.58								+++	
73	33.62						+++			+
75	35.39					+				
76	35.44									+
79	36.27								+++	

Compound	***Ret. time***	***SL***	***ST***	***CA***	***CS***	***PV***	***PS***	***HV***	***SA***	***DC***
82	37.70						++			
83	37.73	+			+++				++	
84	37.93								+	
85	38.79								+	
86	38.80							+	+	
87	39.41						+			++
88	40.41								+++	
89	40.48				+					
91	42.01									++
92	42.18	++	+++	+	++	+	+++	++		++
94	42.89						++			
97	43.45						++			
98	43.53							+++	+	
100	43.96					++				
101	44.72		+++	++	+	+	++	++	++	+
103	45.44								++	
104	45.84						++			
106	46.14							+		
107	46.51						++		+	
108	46.84				+++					
109	47.02						++			
110	47.87				+				++	+
111	48.16						++			
113	48.97		+++							
116	49.68						++			
117	50.76						++			
118	51.56						++			
119	51.64		+++	+++	+	+		+++	+++	+++
120	52.59	++								
121	53.06						+++			
122	53.21				++					
127	54.93						++			
128	54.92									++
129	57.33									+++
132	58.82	++	++	+++	+++	++	++	++	++	++
133	59.80	+								+
135	60.75					+		+		
136	61.43				+		+			
137	62.96									+++

Compound	*Ret. time*	*SL*	*ST*	*CA*	*CS*	*PV*	*PS*	*HV*	*SA*	*DC*
139	64.32	+	+				+			+
140	65.66	+								
141	66.23		+++	++	+	+	++	+	++	++
142	67.00		+++	++	+	+	++	+	++	+
143	67.48								++	
145	67.69					+				
146	68.44		+++	++	+	+	++	+++	++	+
148	69.06									+++
149	70.63	+	+++	++	+	+	+++	+++	++	+
150	70.81									+++
153	71.84	++								+++
155	75.12		+++	+	+	++	++	+	+	+
156	75.83									+
157	76.60						+			
158	76.80	+++								
161	78.62		+++			+		++		
162	79.42		++	++	+	+	+++	++	++	+
164	80.62	++	+++	++	+	+	++	++	++	++
168	82.90		+++	++	+	+	+++	++	++	+
169	84.00	++	+++	++	+++	+	++	+++	++	++
170	84.50	+	+++	+++	+++	+	++	++	++	+++
172	88.34		+++	++	+	+	++	++	++	+
173	88.88	+	+++	++	+	+	++	+	++	+
175	90.84		+++	++	+	++	++	++	++	+
177	93.62		+++	++	+	+	++	++	++	+
179	96.01	+	+++	++	++	+	++	+	++	++
181	99.91		+++	++	+	++	++	+	++	+
182	101.04	+++	+++	+++	+++	++	++	+++	++	++
183	105.73	++	++		++	+		+		
184	106.73		+++			+		++		
185	109.00		+							

All samples contained the following metabolites: tryptophane, benzoic acid, and various non-identified compounds (92, 101, 132, 141, 142, 146, 149, 155, 162, 164, 168, 169, 170, 172, 173, 175, 177, 179, 181, 182). Analyses results for the single investigated species are listed below. The amounts are mean ± SE (% relative peak areas):

SOLANACEAE

Solanum lycopersicum **(tomato)**

All replicates:

	Tryptophane	**16.1 ± 2.9**
	Benzoic acid	**7.99 ± 2.84**
Two replicates:		
	Salicylic acid	3.33 ± 0.63
One replicate:		
	Vanillic acid	1.99
	Ferulic acid	1.09
	Cinnamic acid	**5.73**

Exclusive NI:
120, 133, 140, 158

Solanum tuberosum **(potato)**

All replicates:

	Tryptophane	4.86 ± 0.97
	Benzoic acid	0.84 ± 0.07

Exclusive NI:
113, 185

Capsicum annuum **(potato)**

All replicates:

	Tryptophane	4.47 ± 3.05
	Benzoic acid	1.39 ± 0.49

Exclusive NI:
30, 62

CUCURBITACEAE

Cucumus sativus **(cucumber)**

All replicates:

	Tryptophane	**8.74 ± 1.90**
	Vanillic acid	4.05 ± 1.07
	Benzoic acid	4.02 ± 1.37
	Cinnamic acid	2.70 ± 0.29
Two replicates:		
	Tyrosine	2.08 ± 0.26

Exclusive NI:
28, 45, 56, 60, 89, 108, 122

FABACEAE

Phaseolus vulgaris **(bean)**

Two replicates:

	Tryptophane	**14.2 ± 7.5**
	Benzoic acid	1.91 ± 0.41
One replicate:		
	p-Coumaric acid	3.93

Exclusive NI:
37, 75, 100, 145

Pisum sativum **(pea)**

All replicates:

	Tryptophane	1.24 ± 0.47
	Benzoic acid	1.28 ± 0.46
Two replicates:		
	Pisatin	0.72 ± 0.02

Exclusive NI:
20, 37, 64, 82, 94, 97, 104, 109, 111, 116, 117, 118, 121, 127, 157

POACEAE
***Hordeum vulgare* (barley)**

All replicates:		
	Tyrosine	**12.4 ± 9.6**
	Tryptophane	1.01 ± 0.25
	Benzoic acid	2.01 ± 0.84
Two replicates:		
	Sinapinic acid	0.28 ± 0.07
One replicate:		
	Phenylalanine	0.36 ± 0.00
	p-Coumaric acid	0.84 ± 0.00

Exclusive NI:
14, 26, 31, 55, 106

BRASSICACEAE
***Sinapis alba* (white mustard)**

All replicates:		
	Tyrosine	**9.91 ± 3.10**
	Tryptophane	**6.32 ± 1.09**
	Benzoic acid	3.73 ± 0.99
	Kaempferol derivative 2	1.85 ± 0.58
Two replicates:		
	Phenylacetic acid	0.42 ± 0.14
	Kaempferol derivative 1	0.57 ± 0.01
	Phenylalanine	0.47 ± 0.11

Exclusive NI:
13, 15, 18, 24, 32, 42, 49, 57, 66, 72, 79, 84, 85, 88, 103, 143

APIACEAE
***Daucus carota* (carrot)**

All replicates:		
	Tryptophane	**10.8 ± 4.8**
	Phenylacetic acid	2.50 ± 1.09
	Benzoic acid	4.12 ± 2.60
Two replicates:		
	Ferulic acid	4.73 ± 1.41
One replicate:		
	Vanillic acid	0.57
	Chlorogenic acid	0.71
	Cinnamic acid	1.03

Exclusive NI:
7, 36, 59, 76, 91, 128, 129, 133, 137, 148, 150, 156

3.2 Mycorrhizal colonisation and phosphorus supply (Experiment 2)

3.2.1. Mycorrhizal colonisation

Root colonisation differed between the different plant species, but not between the accessions of one particular species (low standard errors in Table 3). While the roots of barley were not mycorrhized at all (besides of one repetition with 3), the other species showed moderate, tomato with 26 ± 3.27, average, cucumber with 43.67 ± 5.31, and very high, carrot with 70.67 ± 4.99*,* mycorrhization rates.

Table 3: Percentage of root colonisation by *Glomus mossae* (mean ± SE. M1,M2,M3, mycorrhized plant replicates 1, 2 and 3)

	H. vulgare	*S. lycopersicum*	*C. sativus*	*D. carota*
M1	0.00	22.00	37.00	72.00
M2	0.00	26.00	50.00	76.00
M3	3.00	30.00	44.00	64.00
mean	1.00 ± 1.41	26.00 ± 3.27	43.67 ± 5.31	70.67 ± 4.99

3.2.2. GC–MS analysis

All root exudates yielded chromatograms with a total 67 substances found throughout all samples, of which 54 could be identified with the available methods (tentative characterisation according to a comparison of mass spectra and retention time). Metabolites, which couldn't be identified, were named as not identified (NI) in the ongoing text. Table 4 lists all substances detected in the analysed exudate accessions.

Table 4: Substances detected with GC–MS. +, ++ and +++ = found in 1, 2 or all 3 replicates. D=*Daucus carota*, C=*Cucumis sativus*, S=*Solanum lycopersicum*, H=*Hordeum vulgare*, K=control, P=higher phosphorous nutrition/+P, M=mycorrhized. For DK, CK, CP, and HP only samples from two replicates were available.

substance	*Ret. time*	*DK*	*DP*	*DM*	*CK*	*CP*	*CM*	*SK*	*SP*	*SM*	*HK*	*HP*	*HM*
Organic acids													
Oxalic acid	8.73	++	+	+++	++	++	+++	+++	+++	+++	+++	++	+++
Lactic acid	9.30	++	+++	+++	++	++	+++	+++	+++	+++	+++	++	+++
Malonic acid	10.94			+++		++		+++	+++	+++			
Succinic acid	15.18	++	+++	+++	++	++	+++	+++	+++	+++	+++	++	+++
Fumaric acid	16.66				++	++	+++	++			+	+	++
Glutaric acid	18.71			++									
Pyruvic acid	20.97				+	++	+++				+	+	+++
Malic acid	22.18	+	+++	+	+	++	+++			+	+	++	++
Citric acid	34.30	+	+++										
Amino acids and derivatives													
Valine	11.07				++		+++				+++	++	+++
Leucine	13.27			+	+		+++			+	+++	++	+++
Isoleucine	13.89			+	++		+++			+	+++	++	+++
Glycine	14.70								+		+++	+	+
Uracil	15.80									+	+++	++	+++
Threonine	17.93	++	+++	++							+		+
Thymine	18.22										+++	++	+++
γ-aminobutyric acid	23.40		++	+	++	++	+++	+++	++	+++	+++	++	+++

substance	*Ret. time*	*DK*	*DP*	*DM*	*CK*	*CP*	*CM*	*SK*	*SP*	*SM*	*HK*	*HP*	*HM*
Proline	25.23											+	+
Putrescine	30.45							++	+++	+++	+++	+	+
Histamine	32.81				+		++						
Fatty acids													
Glycerol	13.45	+	+++	+++	++	++	+++	+++	+++	+++	+++	++	+++
Glyceric acid	15.88	++	+++	+++	++	++	+++	+++	+				
Palmitic acid	41.06					++		+	+	+	+++	+	+++
Stearic acid	46.75					+		+		+	+++	+	++
Sugar derivatives and - acids													
Threitol	22.80	++	+++	++		++	+++						
Threonic acid	24.38	++	++	+++	++	++	+++	+++	++	+++	+++		++
Ribitol	30.67	+			++	++	+++						
Ribonic acid	31.46			+++		++	+++				+		
2-Keto-l-gluconic acid	32.08		++	+++		++	+++		+		+++	+	+
scyllo-Inositol	35.89			+++									
Glucopyranose	39.39	+	++	+++		++				++			+
Gulonic acid	39.66	++	+++	+++		+	+++		+				
myo-Inositol	42.22	++	+++	+++	++	++	+++	+++	+++	+++	+++	++	+++
Mannitol/Sorbitol	43.43	+	+++	+++				+++	+	++			
Galacturonic acid	49.06	+	+++	+++					+	+			+
Anhydrosorbitol	57.77					+							
Sugars													
Xylose	28.61	++	+++	+									
Arabinose	29.57		+	+++		++	+++						
Rhamnose	30.41			++									
Mannose	33.60	+	+++	++									
Fructose	34.10	++	+++	++		+		+++	+++	+++			
Galactose	36.50	+	+++	+++	++	++	+++	+++	++	+++			
Glucose	36.89	++	+++	+++	++	++	+++	++	++	+++			
Cellobiose	55.79					+	++						
Sucrose	57.03	++	+++	++		+	+	+++	++	++			
Maltose	58.38	+		+	+	++	+++		++	+			
Melibiose	64.64		+	+	++	++	+++	+++	++	+++			
Melizitose	68.40						+	+++	+	+++			

substance	*Ret. time*	*DK*	*DP*	*DM*	*CK*	*CP*	*CM*	*SK*	*SP*	*SM*	*HK*	*HP*	*HM*
Maltotriose	73.12	++	+++	+++									
Aromatic compounds and shikimic acid pathway derivatives													
Benzoic acid	12.17	++	+++	+++	++	++	+++	+++	+++	+++	+++	++	+++
4-Hydroxy-benzoic acid	26.85			++		++			+				
Shikimic acid	33.99					++							
Quinic acid	35.16	+	+++	+++				+++	+++	+++			
Not identified substances (NI)													
1	9.55	+	++	+++	++	++	+++	+	+++		+	++	++
2	11.17	+	+	+++									
3	11.75	+	+++	+++	++		+	+++					
4	14.41							++		+			
6	18.05				++	++	+++						
9	25.50			+++		++		+					
10	28.39							+					
11	28.82			+++		++			+				
12	28.94										++	+	+++
17	34.13			++									
18	38.40	++		++			+++	++		++	+		+
19	40.77		++										
24	54.74			+									

Analyses results for the single investigated treatments and species are listed below. The amounts are mean ± SE (% relative peak areas):

Daucus carota **(carrot)**

Control

All replicates:

Oxalic acid	2.96 ± 1.17
Lactic acid	1.90 ± 1.05
Succinic acid	**32.9 ± 12.6**
Threonic acid	0.53 ± 0.27
Threonine	0.40 ± 0.21
***myo*-Inositol**	**5.22 ± 2.82**
Threitol	0.25 ± 0.07
Glyceric acid	0.21 ± 0.07
Gulonic acid	1.97 ± 1.06
Xylose	0.97 ± 0.58
Glucose	2.86 ± 1.74
Fructose	**21.3 ± 14.8**
Sucrose	0.45 ± 0.24
Maltotriose	0.40 ± 0.16
Benzoic acid	4.29 ± 2.25
NI 18	0.52 ± 0.31

One replicate:

	Malic acid	0.32
	Citric acid	**11.1**
	Glycerol	2.73
	Glucopyranose	0.57
	Ribitol	0.51
	Galacturonic acid	0.63
	Mannitol	0.63
	Galactose	**17.3**
	Mannose	**5.9**
	Maltose	0.23
	Quinic acid	3.14
	NI 1	0.94
	NI 2	0.95
	NI 3	0.94

+P

All replicates:		
	Malic acid	0.13 ± 0.01
	Citric acid	4.42 ± 1.45
	Lactic acid	0.24 ± 0.03
	Succinic acid	**14.9 ± 2.8**
	Threonine	0.23 ± 0.08
	Glycerol	2.68 ± 0.61
	myo-Inositol	3.28 ± 0.43
	Galacturonic acid	0.15 ± 0.01
	Glyceric acid	0.14 ± 0.05
	Mannitol	1.40 ± 0.52
	Gulonic acid	2.13 ± 0.84
	Xylose	0.39 ± 0.06
	Galactose	0.11 ± 0.06
	Glucose	**12.8 ± 2.4**
	Mannose	1.77 ± 0.66
	Fructose	**48.8 ± 3.9**
	Sucrose	0.14 ± 0.06
	Maltotriose	0.16 ± 0.02
	Benzoic acid	0.08 ± 0.02
	Quinic acid	1.43 ± 0.19
	NI 3	0.20 ± 0.03
Two replicates:		
	γ-aminobutyric-acid	0.23 ± 0.17
	Threonic acid	0.12 ± 0.05
	Glucopyranose	**5.24 ± 2.23**
	2-Keto-l-gluconic-acid	0.11 ± 0.02
	NI 1	0.07 ± 0.02
	NI 19	0.18 ± 0.01
One replicate:		
	Oxalic acid	0.17
	Arabinose	0.08
	Melibiose	0.06
	NI 2	0.05

+M

All replicates:		
	Oxalic acid	3.32 ± 0.20
	Lactic acid	1.89 ± 0.38
	Succinic acid	**34.4 ± 6.9**
	Malonic acid	1.31 ± 0.34
	Ribonic acid	0.29 ± 0.06
	Glycerol	1.60 ± 0.64
	Threonic acid	0.66 ± 0.16

	***myo*-Inositol**	**20.0 ± 0.9**
	Galacturonic acid	0.97 ± 0.15
	Glyceric acid	0.40 ± 0.05
	Mannitol	2.54 ± 0.28
	2-keto-l-gluconic acid	1.08 ± 0.26
	Scyllo-inositol	0.91 ± 0.13
	Glucopyranose	0.76 ± 0.20
	Gulonic acid	0.89 ± 0.11
	Arabinose	0.49 ± 0.07
	Galactose	1.08 ± 0.17
	Glucose	1.14 ± 0.29
	Maltotriose	**5.44 ± 1.39**
	Benzoic acid	3.96 ± 1.48
	Quinic acid	3.30 ± 1.72
	NI 1	0.76 ± 0.06
	NI 2	0.91 ± 0.13
	NI 3	2.40 ± 0.26
	NI 9	0.42 ± 0.08
	NI 11	1.89 ± 0.73
Two replicates:		
	Glutaric acid	0.35 ± 0.01
	Threonine	0.77 ± 0.08
	Threitol	0.48 ± 0.03
	Rhamnose	1.41 ± 0.11
	Mannose	0.27 ± 0.01
	Fructose	1.22 ± 0.13
	Sucrose	0.64 ± 0.37
	4-Hydroxybenzoic-acid	0.37 ± 0.03
	NI 17	0.88 ± 0.44
	NI 18	0.28 ± 0.07
One replicate:		
	Malic acid	0.20
	γ-aminobutyric-acid	1.71
	Leucine	0.35
	Isoleucine	2.11
	Xylose	0.54
	Maltose	0.68
	Melibiose	0.16
	NI 24	0.28

Cucumus sativus (cucumber)

Control

All replicates:		
	Oxalic acid	0.60 ± 0.09
	Lactic acid	1.67 ± 0.06
	Succinic acid	**58.9 ± 2.1**
	Fumaric acid	0.38 ± 0.05
	γ-aminobutyric-acid	1.68 ± 0.19
	Valine	0.84 ± 0.17
	Isoleucine	0.78 ± 0.18
	Glycerol	0.65 ± 0.03
	Threonic acid	3.34 ± 0.00
	***myo*-Inositol**	**22.1 ± 4.1**
	Glyceric acid	1.11 ± 0.35
	Ribitol	0.78 ± 0.17
	Galactose	0.68 ± 0.20
	Glucose	0.71 ± 0.42
	Melibiose	0.85 ± 0.10
	Benzoic acid	0.25 ± 0.05

	NI 1	0.26 ± 0.04
	NI 3	0.20 ± 0.04
	NI 6	2.55 ± 0.62
One replicate:		
	Malic acid	1.73
	Pyruvic acid	0.35
	Leucine	0.56
	Histamine	0.60
	Maltose	0.22

+P

All replicates:		
	Oxalic acid	**5.5 ± 0.1**
	Lactic acid	2.87 ± 0.89
	Succinic acid	**40.8 ± 0.2**
	Fumaric acid	0.27 ± 0.06
	Malonic acid	0.21 ± 0.02
	Malic acid	**6.42 ± 3.73**
	Pyruvic acid	0.38 ± 0.12
	Ribonic acid	0.44 ± 0.13
	γ-aminobutyric-acid	0.16 ± 0.03
	Glycerol	0.34 ± 0.08
	Palmiticacid	0.35 ± 0.10
	Threitol	0.24 ± 0.00
	Threonic acid	2.33 ± 0.41
	***myo*-Inositol**	**23.4 ± 2.6**
	Glyceric acid	0.69 ± 0.16
	Ribitol	0.47 ± 0.03
	2-keto-l-gluconic acid	0.20 ± 0.00
	Glucopyranose	1.40 ± 0.43
	Arabinose	0.20 ± 0.02
	Galactose	0.40 ± 0.01
	Glucose	4.43 ± 2.30
	Maltose	0.41 ± 0.17
	Melibiose	0.59 ± 0.07
	4-Hydroxy-benzoic acid	0.23 ± 0.01
	Benzoic acid	0.40 ± 0.12
	Shikimic acid	0.64 ± 0.23
	NI 1	0.24 ± 0.01
	NI 6	2.36 ± 0.45
	NI 9	0.17 ± 0.01
	NI 11	0.15 ± 0.02
One replicate:		
	Stearic acid	0.37
	Anhydrosorbitol	1.37
	Gulonic acid	0.30
	Fructose	0.95
	Cellobiose	0.24
	Sucrose	0.47

+M

All replicates:		
	Oxalic acid	1.29 ± 0.26
	Lactic acid	0.81 ± 0.03
	Fumaric acid	0.09 ± 0.00
	Pyruvic acid	0.40 ± 0.05
	Malic acid	0.22 ± 0.06
	Ribonic acid	0.28 ± 0.01
	Succinic acid	**64.9 ± 1.1**
	Leucine	0.77 ± 0.22
	Isoleucine	0.92 ± 0.08

	Valine	1.13 ± 0.14
	γ-aminobutyric-acid	1.11 ± 0.30
	Glycerol	0.48 ± 0.04
	Threonic acid	2.50 ± 0.28
	***myo*-Inositol**	**13.6 ± 1.1**
	Threitol	0.30 ± 0.05
	Glyceric acid	0.30 ± 0.08
	Ribitol	1.31 ± 0.22
	2-keto-l-gluconic acid	0.20 ± 0.02
	Gulonic acid	0.30 ± 0.02
	Arabinose	0.34 ± 0.02
	Galactose	0.33 ± 0.04
	Glucose	0.90 ± 0.20
	Maltose	0.63 ± 0.02
	Melibiose	0.53 ± 0.07
	Benzoic acid	2.65 ± 0.45
	NI 1	0.28 ± 0.01
	NI 6	2.02 ± 0.27
	NI 18	0.62 ± 0.03
Two replicates:		
	Histamine	0.18 ± 0.03
	Cellobiose	0.19 ± 0.04
One replicate:		
	Sucrose	0.92
	Melizitose	0.23
	NI 3	0.24

Solanum lycopersicum (tomato)

Control

All replicates:		
	Oxalic acid	1.05 ± 0.24
	Lactic acid	**6.24 ± 2.75**
	Succinic acid	**20.2 ± 6.8**
	Malonic acid	0.40 ± 0.14
	γ-aminobutyric-acid	**7.23 ± 2.51**
	Glycerol	4.08 ± 0.97
	Threonic acid	1.11 ± 0.22
	***myo*-Inositol**	**39.9 ± 3.0**
	Glyceric acid	0.25 ± 0.02
	Mannitol	0.86 ± 0.30
	Fructose	0.34 ± 0.02
	Galactose	0.56 ± 0.08
	Sucrose	0.37 ± 0.11
	Melizitose	1.15 ± 0.37
	Melibiose	2.62 ± 0.70
	Benzoic acid	1.04 ± 0.42
	Quinic acid	4.00 ± 0.03
	NI 3	1.55 ± 0.44
Two replicates:		
	Fumaric acid	0.22 ± 0.01
	Putrescine	0.53 ± 0.14
	Glucose	0.18 ± 0.04
	NI 4	1.70 ± 0.59
	NI 18	0.48 ± 0.10
One replicate:		
	Palmitic acid	0.63
	Stearic acid	0.32
	NI 1	0.27
	NI 9	2.96

	NI 10	0.42

+P

All replicates:		
	Oxalic acid	3.84 ± 1.99
	Lactic acid	4.51 ± 0.39
	Succinic acid	1.68 ± 0.30
	Malonic acid	0.71 ± 0.23
	Putrescine	1.17 ± 0.37
	Glycerol	**17.5 ± 9.8**
	myo-Inositol	**24.6 ± 10.0**
	Fructose	4.33 ± 0.99
	Benzoic acid	**20.0 ± 13.6**
	Quinic acid	4.03 ± 2.34
	NI 1	1.26 ± 0.25
Two replicates:		
	γ-aminobutyric-acid	**5.6 ± 2.7**
	Threonic acid	0.48 ± 0.08
	Galactose	1.37 ± 0.18
	Glucose	0.63 ± 0.19
	Sucrose	3.16 ± 1.62
	Maltose	0.40 ± 0.16
	Melibiose	**7.63 ± 2.61**
One replicate:		
	Glycine	1.72
	Palmiticacid	0.32
	Glyceric acid	0.30
	Galacturonic acid	2.93
	Mannitol	0.38
	2-keto-l-gluconic acid	0.28
	Gulonic acid	1.96
	Melizitose	0.13
	4-hydroxybenzoic-acid	0.47
	NI 11	0.47

+M

All replicates:		
	Oxalic acid	1.74 ± 0.23
	Lactic acid	3.56 ± 0.69
	Succinic acid	**11.3 ± 0.6**
	Malonic acid	0.24 ± 0.01
	Putrescine	1.20 ± 0.41
	γ-aminobutyric-acid	**7.7 ± 1.7**
	Glycerol	**6.85 ± 4.66**
	***myo*-Inositol**	**41.8 ± 2.6**
	Threonic acid	0.84 ± 0.49
	Galactose	0.59 ± 0.13
	Glucose	0.40 ± 0.06
	Fructose	4.69 ± 2.79
	Melibiose	4.69 ± 2.79
	Melizitose	0.69 ± 0.22
	Benzoic acid	**8.33 ± 2.46**
	Quinic acid	1.78 ± 0.16
Two replicates:		
	Glucopyranose	0.26 ± 0.06
	Mannitol	1.56 ± 0.70
	Sucrose	0.93 ± 0.44

One replicate:

Malic acid	0.29
Leucine	1.05
Isoleucine	1.93
Uracil	0.23
Palmitic acid	1.03
Stearic acid	0.58
Galacturonic acid	0.45
Maltose	0.30
NI 4	1.77

Hordeum vulgare (barley)

Control

All replicates:

Oxalic acid	**5.66 ± 2.41**
Lactic acid	**8.37 ± 0.24**
Succinic acid	**29.8 ± 9.3**
Leucine	**7.01 ± 1.19**
Thymine	0.33 ± 0.08
Isoleucine	**7.84 ± 1.52**
Glycine	2.64 ± 1.60
Valine	**8.52 ± 1.68**
Uracil	0.89 ± 0.18
γ-aminobutyric-acid	**12.3 ± 4.2**
Putrescine	0.65 ± 0.20
Glycerol	1.28 ± 0.53
Palmitic acid	0.68 ± 0.06
Stearic acid	0.39 ± 0.02
Threonic acid	0.55 ± 0.27
myo-Inositol	4.48 ± 0.81
2-Keto-l-gluconic acid	0.36 ± 0.06
Benzoic acid	3.94 ± 1.45

Two replicates:

NI 12	0.41 ± 0.04

One replicate:

Fumaric acid	2.18
Pyruvic acid	0.53
Malic acid	**7.5**
Ribonic acid	0.30
Threonine	0.64
NI 1	0.59
NI 18	0.42

+P

All replicates:

Oxalic acid	2.55 ± 1.18
Lactic acid	2.55 ± 0.32
Succinic acid	**66.8 ± 4.2**
Malic acid	0.75 ± 0.43
Leucine	1.31 ± 0.35
Thymine	0.16 ± 0.02
Isoleucine	1.80 ± 0.16
Valine	2.24 ± 0.19
Uracil	0.59 ± 0.01
γ-aminobutyric-acid	2.54 ± 0.18
Glycerol	0.80 ± 0.38
myo-Inositol	3.24 ± 0.48
Benzoic acid	**8.51 ± 5.38**

	NI 1	0.13 ± 0.01
One replicate:		
	Fumaric acid	0.16
	Pyruvic acid	0.69
	Glycine	**7.28**
	Proline	0.19
	Putrescine	0.13
	Palmitic acid	1.85
	Stearic acid	1.20
	2-Keto-l-gluconic acid	0.20
	NI 12	0.24
+M		
All replicates:		
	Oxalic acid	**7.96 ± 3.16**
	Lactic acid	2.94 ± 0.52
	Succinic acid	**61.0 ± 3.8**
	Pyruvic acid	0.96 ± 0.62
	Leucine	2.06 ± 0.77
	Thymine	0.30 ± 0.06
	Isoleucine	2.78 ± 0.78
	Valine	3.16 ± 0.43
	Uracil	0.79 ± 0.12
	γ-aminobutyric-acid	3.99 ± 1.75
	Glycerol	0.38 ± 0.09
	Palmiticacid	1.03 ± 0.49
	Myo-inositol	3.43 ± 0.78
	Benzoic acid	2.35 ± 0.51
	NI 12	0.55 ± 0.28
Two replicates:		
	Fumaric acid	0.40 ± 0.21
	Malic acid	3.81 ± 2.48
	Stearic acid	0.83 ± 0.31
	Threonic acid	0.61 ± 0.28
	NI 1	0.69 ± 0.20
One replicate:		
	Threonine	0.45
	Glycine	2.40
	Proline	0.52
	Putrescine	0.54
	2-Keto-l-gluconic acid	0.41
	Glucopyranose	0.62
	Galacturonic acid	0.45
	NI 18	0.54

3.2.3 HPLC–UV analysis

All root exudates analysed with HPLC yielded chromatograms with a total of 171 substances found throughout all samples, of which 15 could be identified with the available methods (tentative characterisation according to a comparison of UV spectra libraries and retention time). Metabolites which couldn't be identified were named as not identified (NI) in the ongoing text. For all of the treatments three replicates were prepared, but due to different problems with the collection of the exudates, the sample preparation, or the measurements, for some treatments just analyses from two or even one replicate are available. Table 5 lists all substances detected in the analysed exudate accessions.

Table 5: Substances detected with HPLC. +, ++ and +++ = found in one, two or all 3 replicates. D=*Daucus carota*, C= *Cucumis sativus*, S=*Solanum lycopersicum*, H=*Hordeum vulgare*, K=control, P=higher phosphorous nutrition/ +P, M=mycorrhized. For CP, SM, and HP only two replicates were analysed, for DK only one.

Compound		***DK***	***DP***	***DM***	***CK***	***CP***	***CM***	***SK***	***SP***	***SM***	***HK***	***HP***	***HM***
Amino acids													
Tyrosine	4.91					++	+				++		
Phenylalanine	7.66						+	++	++	++	++		+
Tryptophane	14.28		+	++	+	+	++	+		++	+	++	+
Aromatic acids													
Vanillic acid	18.22		+										
Phenylacetic acid	20.03	+	+	+++									+
Syringic acid	20.47				+++	++	+						
p-Coumaric acid	27.48		++		+								
Benzoic acid	30.11	+	+++	+++	+++	++	+++	+++	+	++	+++	++	+++
Ferulic acid	30.72	+	+++	+++									
p-Coumaric acid derivative	31.06		+		++		+						
Salicylic acid	33.65	+	++				+	+++		++			
Sinapinic acid (35.70)	35.70				++	+	+						
Chlorogenic acid	37.03		+++	+									
Cinnamic acid	42.89		+		+	+	+	+++		++		+	+
Cinnamic acid derivative	52.46		++										
Not identified substances (NI)													
1	2.67	+	+++	+++	+++	++	+++	++	+	++	+	++	+++
2	2.84										+++		+
5	4.34										+++	++	+++
6	4.95		++	+++						+			
7	5.82				+++	++	+	++		++			+
8	7.90	+		+									
9	8.64	+	++	+++									
10	8.79				+++	++	+++						
11	10.74		+		+++	++	+			+			
12	11.59											+	+++
13	13.31	+	++	++									
14	14.84	+	+++	+++	+++	++	+++				+	+	++
16	16.35											++	++
17	16.68				++	++	+						

Compound		***DK***	***DP***	***DM***	***CK***	***CP***	***CM***	***SK***	***SP***	***SM***	***HK***	***HP***	***HM***
18	16.77			+++									
20	18.03				+++	++	+++						
21	18.12	+		+++									
22	18.20									+		++	+++
23	19.22											+	++
24	19.42				++	++	+						
25	20.63										+	++	+
27	20.91	+	++	+++	+	+					+		+
28	21.29				+++	+	+						
29	22.17			+						+			
30	22.22				+								
31	22.60					++	+++						+
32	22.63											++	+
33	22.64	+	+++	+++	++					+			
34	23.18							++		++		+	
35	23.25			+++									
37	23.42		++										
39	23.83										+	++	++
40	23.98	+	+	++		+	+	++	+	+			
41	24.28			++									
43	24.78				++	++	+						
44	24.95					+				++			
45	25.03		+	+++									
46	25.96	+	+	++									
47	26.05				+	+	+						
48	26.15	+	+	+				++		+			
50	26.60	+	+++	+++									
51	26.92				+++	++	++	+++	+	++	+	++	+++
53	27.54										+	++	
54	27.77	+		+++									
55	27.94				+++	++	+						++
56	28.53				+++	++	+						
58	28.58		++	+++				++					
59	29.11	+		+++				+					
60	29.47				+++	++	++					+	+
61	30.49				+	+	+						
63	30.90											+	+
65	31.43									+			+
67	31.82			++									

Compound		***DK***	***DP***	***DM***	***CK***	***CP***	***CM***	***SK***	***SP***	***SM***	***HK***	***HP***	***HM***
68	32.23	+	+++	++	+++	++	+++						
70	32.98	+		++									
71	33.39				+++	++							
73	33.62		+++	+++							+	++	+++
74	34.60	+		+++	++	+	+					++	
75	35.39			+	++		+						
76	35.44		++										
77	35.54									++			++
78	35.83	+	+	+									
80	36.47				++	+		+					
81	37.01										+		+
82	37.70				+++	++	+					++	+
83	37.73			+++									
84	37.93		+++										
85	38.79		+	+	++	++	+					+	++
87	39.41	+	+++	+++	++	++	++						
88	40.41				+++	++	+++						
89	40.48	+	+++	+++							++	++	+++
90	41.48		+	+++									
91	42.01				+++	++	+++						
92	42.18	+	+	+++				++		+		++	+++
93	42.54			++									
95	43.27.				+++	+	+				+++	++	++
96	43.35	+		+++									
99	43.91				++		+						
101	44.72	+	+++	+++	+++	++	+++	++	+	+	+	++	+++
102	45.20							+++					
104	45.84		+++		++		+						
105	45.87	+		+++									
106	46.14											+	
107	46.51		+										
108	46.84				+++	++	+						
110	47.87	+	+++	+++	+++	++	+	++		+	+	++	+++
112	48.75			+						+			
114	49.06				++	+	+						
115	49.62			++									
117	50.76						+					+	
118	51.56	+	+++		+++	++	+	++	+	++	+	++	+++
119	51.64		+++	+++									

Compound		DK	DP	DM	CK	CP	CM	SK	SP	SM	HK	HP	HM
120	52.59			+++	+	+	+						
122	53.21			+	+++		+						
123	54.29			+++									
124	54.50						+			+			+
125	54.79			+++									
126	54.92				+		+						
129	57.33		+++	++									
130	57.39			+	+		+		+				
131	58.11			+++									
132	58.82	+	++		+++	++	+++	+++	+++	++	+++	++	+++
133	59.80							+			+		
134	60.51			++	+		+						
135	60.75							+	++	+			
136	61.43			+	++	++	+						
137	62.96	+	++	+++	+		+						
138	63.58				++	++	++						
139	64.32	+		+	+						+	+	
140	65.66						+		+			+	
141	66.23	+	+++	+	++	+		++	+	+	+	++	+++
142	67.00	+	+++	+++	+	+		++	+	+	+	+	+++
143	67.48	+	+++										
144	67.50			+++									
145	67.69				+	++		+	+	+		+	+++
146	68.54	+		+	+++	++	+	+		+	+	+	+++
147	68.95			+++									
148	69.06	+	+++										
149	70.36	+			++		++	+	++	+	+	+	
150	70.81	+	+++	+++									
151	71.44				+		++	+	++	+	++		
152	71.73					+	+						
153	71.84	+	+++	+++						+			
154	74.98		++	++									
155	75.12	+		+++	++	+					+++	+	++
156	75.83		++	+		+	+	+	++	+		+	
157	76.60	+	+++	+++									
158	76.80				++	+	+	++	+	+	+	++	+
159	77.75				++	+		++	+	+	+	++	+++
160	77.97	+	+++	++									
161	78.62	+		+	++		++	+	+	++		+	+

Compound		*DK*	*DP*	*DM*	*CK*	*CP*	*CM*	*SK*	*SP*	*SM*	*HK*	*HP*	*HM*
162	79.42			+									
163	79.92		+	++									
164	80.62			+	+	+	+	++	+++	++	+++	++	++
165	81.05		++										
166	82.09	+	+++	++	+	+	+	++			+	++	++
167	82.73	+	++										
168	82.90			++						+		+	
169	84.00	+	++	+	+	+		+++	+	+	+++	+	+++
170	84.50	+	++	+	+	+		++	+	+	+	++	+++
171	85.78			++		+		+		+	+	+	++
172	88.34	+	+++	+	+	+		++	+	++	++	+	+++
173	88.88	+		+		+		+	++		++		++
174	89.72		+++	+	+	+	++	+++	++	+		+	
175	90.84	+	++	+		+		++	+	+	+++	+	+++
176	91.84			+				++		+	+	+	
177	93.62	+	++	+	++	+		+	+	+	+++	+	+++
178	95.55				+	+	+						
179	96.01			+		+		+		+	+	+	++
180	98.72			+		++	+			+	+	+	
181	99.91	+	++	+	++	+		++	+	++	+	+	+++
182	101.40	+	++	++	++		+	+++	+++	++	+++	++	+++
183	105.73			+	+	+		+++		+	+	+	++
184	106.73			++	+			+++		++		+	
185	109.00									+	+	+	

All samples contained the following metabolites: benzoic acid and various non-identified compounds (1, 101, 110, 118, 132, 141, 142, 169, 170, 172, 177, 181, 182). Specific analytes for the respective accessions are listed below.

Daucus carota **(carrot)**

Control

One replicate:

Phenylacetic acid	0.54
Benzoic acid	**17.0**
Ferulic acid	1.12

Exclusive NI:

8, 21, 54, 70, 96, 105, 143, 148, 167

+P

All replicates:

Benzoic acid	**11.3 ± 3.3**
Ferulic acid	4.07 ± 1.00

	Chlorogenic acid	**5.06 ± 1.49**
Two replicates:		
	p-Coumaric acid	0.39 ± 0.04
	Cinnamic acid derivative	0.57 ± 0.01
One replicate:		
	Tryptophane	0.39
	Vanillic acid	0.34
	Phenylacetic acid	0.78
	p-Coumaric acid derivative	1.16
	Cinnamic acid	0.36

Exclusive NI:
37, 45, 76, 84, 90, 107, 119, 129, 148, 154, 163, 165, 167.

+M

All replicates:		
	Benzoic acid	2.71 ± 1.11
	Ferulic acid	3.01 ± 0.34
	Phenylacetic acid	0.51 ± 0.12
Two replicates:		
	Tryptophane	0.19 ± 0.06
One replicate:		
	Chlorogenic acid	0.25

Exclusive NI:
8, 18, 21, 29, 35, 41, 45, 54, 67, 70, 83, 90, 93, 96, 105, 112, 115, 119, 123, 125, 129, 131, 143, 147, 154, 162, 163

Cucumus sativus (cucumber)

Control

All replicates:		
	Syringic acid	0.41 ± 0.02
	Benzoic acid	**5.26 ± 3.35**
Two replicates:		
	Sinapinic acid	0.61 ± 0.05
	p-Coumaric acid derivative	0.47 ± 0.00
One replicate:		
	Tryptophane	1.37
	p-Coumaric acid	0.14
	Cinnamic acid	0.35

Exclusive NI:
30, 71, 99, and 126.

+P

All replicates:		
	Tyrosine	0.34 ± 0.05
	Syringic acid	1.25 ± 0.64
	Benzoic acid	**6.64 ± 4.31**
One replicate:		
	Sinapinic acid	0.68 ± 0.24
	tryptophane	0.36 ± 0.13
	Cinnamic acid	0.50 ± 0.18

Exclusive NI:
44, 71, 152.

+M

All replicates:		
	Benzoic acid	**5.57 ± 1.97**
Two replicates:		

	Tryptophane	2.75 ± 0.74
One replicate:		
	Sinapinic acid	0.36
	Tyrosine	0.95
	Phenylalanine	**6.84**
	Syringic acid	0.46
	Coumaric acid derivative	0.56
	Cinnamic acid	0.70

Exclusive NI:
99, 117, 126, and 152.

Solanum lycopersicum (tomato)

Control

All replicates:		
	Benzoic acid	**9.71 ± 3.74**
	Cinnamic acid	0.74 ± 0.17
Two replicates:		
	Phenylalanine	**4.99 ± 3.15**
One replicate:		
	Tryptophane	0.74

Exclusive NI:
102, 133

+P

Two replicates:		
	Phenylalanine	**10.4 ± 0.02**
One replicate:		
	Benzoic acid	**19.9 ± 5.4**

+M

All replicates:		
	Phenylalanine	**4.31 ± 2.70**
	Tryptophane	2.60 ± 1.51
	Benzoic acid	**11.4 ± 5.8**
	Salicylic acid	1.29 ± 0.21
	Cinnamic acid	1.74 ± 0.50

Exclusive NI:
29, 44, 65, 77, 112

Hordeum vulgare (barley)

Control

All replicates:		
	Benzoic acid	**11.1 ± 3.8**
Two replicates:		
	Tyrosine	1.87 ± 1.08
	Phenylalanine	**6.31 ± 0.00**
One replicate:		
	Tryptophane	**6.91**

Exclusive NI:
2, 53, 81, 133

+P

Two replicates:

	Tryptophane	1.84 ± 1.08
	Benzoic acid	**11.6 ± 3.7**
One replicate:		
	Cinnamic acid	1.36

Exclusive NI:
12, 16, 23, 53, 63, 106, 117

+M

All replicates:		
	Benzoic acid	**17.8 ± 0.8**
One replicate:		
	Phenylalanine	0.19
	Tryptophane	2.84
	Phenylacetic acid	0.42
	Cinnamic acid	**6.33**

Exclusive NI:
2, 12, 16, 23, 63, 65, 77, 81

3.3 Benzoic acid seasonyal dynamics (Experiment 3)

The root exudates analysed with HPLC–UV yielded chromatograms with a total of 120 substances found throughout all samples, of which 10 could be identified with the available methods (tentative characterisation according to a comparison of UV spectra and retention time). Metabolites which couldn't be identified were named as not identified (NI) in the ongoing text. Table 6 lists all substances detected in the analysed exudate accessions. For all species and seasons three replicates were prepared.

Table 6: Metabolites detected by HPLC; +, ++ and +++ = found in one, two or all 3 replicates; T=*Taxus baccata*, D=*Digitalis purpurea*, B= *Begonia sutherlandii* . S=summer, W=winter. (S), analytes characteristic for summer accessions (at least detected in two species); (W), analytes characteristic for winter accessions (at least detected in two species).

Compound	*Ret. time*	*TS*	*TW*	*DS*	*DW*	*BS*	*BW*
Aromatic acids							
Benzoic acid	29.20	+++	+++	++	+++	+++	+++
Ferulic acid	30.47	+					
Chlorogenic acid 1	35.06		+	++	+		
Chlorogenic acid 2	35.74		+		+		
Cinnamic acid	42.15		+				
Cinnamic acid derivative	51.56	++					
Aromatic NI1	55.57	+++		+++		+++	
Aromatic NI2	57.50		+				
Flavanoids							
Quercetin glycoside	41.62				+		+
Fatty acid derivatives							
Polyacetylene	87.99	++					
Not identified substances (NI)							
1	2.61	+++	+++	+++	+++	+++	+++

Compound	*Ret. time*	*TS*	*TW*	*DS*	*DW*	*BS*	*BW*
2	3.04	+				+	
3 (W)	3.86		+++		+++		++
4	4.87			+++	+		
6	6.63			+++	++		
7	6.82					+	
8	7.18	+	+				+
9	8.19		+	++			+
11	10.12		+		+		
12	11.61		+				
13 (W)	12.86		+		+		+
14	13.47			++		+	
15	14.30		+				
16	15.18						+
17 (S)	17.31	++		++			
18	17.48		+				+
19	18.87		+	++			+
20	19.22		+				
21	19.82	+	+	++	+		
22	20.55						++
23	21.46	++	+++	+	++	++	+
24	22.44			++			
25	22.63	+	+	+		+	+
26	23.07					+	+
27	23.25	++	+++	+	+		++
28	24.51		+	+++			
29 (W)	24.80		+				+
30	25.69						+
31	25.95	+		++			
32 (W)	26.77		+++		+		++
33 (W)	27.99				+		+
34	28.30	+	+				
35	30.07						+
36	30.23		+++		+		
37	30.99		+			+	
38	31.34	+	+	+			
39	32.66	+	++			+	
40	33.39		++	+		+	+
41	33.54		+				
42	34.28	++	+				

Compound	*Ret. time*	*TS*	*TW*	*DS*	*DW*	*BS*	*BW*
43	36.37	++	+	+			+
44 (W)	37.19		+		+		+
45	38.53		+	+++			
46	39.45	++	+		+	+	+
47 (W)	39.37		+				+
48	40.78	+++		+			
49	41.54		+				
50	41.94	+					
52	43.19			++			
53 (W)	43.74		++		+		+++
54 (S)	44.44	+++	+	+++	+	+	
55	45.10		+				
56 (W)	45.89		+		++		+
57	46.44		+				
58	46.45			++			
59 (W)	47.18		+++		+		+
60	47.92	+++					
61	47.93			++			
62 (W)	47.96		+				+
63 (W)	49.83		+		++		
64	50.79		+++	+	++	++	+++
65	51.34		+				
66	51.87			+			
67 (W)	51.88		+				+
68 (W)	52.58		+				+
69	53.08		+				
70	53.15						+
71	54.26		+				
72 (W)	55.88		+				+
73	58.44	+++	+				
75 (W)	59.80		+				+
76	60.35			++			
77	61.39	++					
78 (W)	63.45		++		++	+	++
79 (S)	64.25	+++	+	+++			
80	62.75		+++		++	+	+
81 (W)	66.47		+++		+		++
82 (W)	67.44		++		+		
83 (S)	67.90	+++		+		+++	

Compound	***Ret. time***	***TS***	***TW***	***DS***	***DW***	***BS***	***BW***
84	68.25		+				
85 (W)	69.30		++		+		
86 (W)	70.67		+		+		
87	72.49	+++					
88	73.40		+				
89	74.26		+			++	+
90 (W)	74.51		+		+		
91	75.02		+				
92	77.26	++					
93	78.80	+		+	+		
94	80.68	++	+++		+		+++
95	81.21	++	+			++	++
96 (W)	81.91		+		+		++
97	83.43		++	+	+++	+++	+++
98	83.95	+	+++	+	+++	+	+++
99	85.83		+				
100 (S)	86.19	+		++			
101 (W)	87.81		++		+		+
102 (W)	88.50		+				
103 (W)	90.42		++	+	+		+
104	91.25	+					
105	91.64			++			
106	92.71	+	+			+	
107 (W)	93.13		++	+	++		+
108	94.03	+++					
109 (W)	95.06		++		+++		+++
110	97.63						++
111	99.70	+++	+++	+++	++		+
112	100.96		++	++	+++	+++	+++
113 (W)	106.75		+		+		
114	108.54				++		
116	115.43		+				
117	118.53			++			
118 (W)	119.09		+++		+		+

All samples contained the following metabolites and various non-identified compounds (1, 23, 98). Specific analytes for the respective accessions are listed below.

Taxus baccata (common yew)

Summer

All replicates:

	Benzoic acid	**4.48 ± 1.22**
	Aromatic unknown 1	**8.09 ± 0.91**
Two replicates:		
	Cinnamic acid derivative	3.20 ± 1.05
One replicate:		
	Ferulic acid	3.81
	Polyacetylene	4.78

Exclusive NI:
50, 77, 87, 92, 104, 108

Winter

All replicates:

	Benzoic acid	**1.29 ± 0.24**
One replicate:		
	Chlorogenic acid 1	1.48
	Chlorogenic acid 2	1.22
	Cinnamic acid	2.00
	Aromatic unknown 1	4.30

Exclusive NI:
12, 15, 65, 69, 71, 84, 88, 91, 99, 102 and 116.

Digitalis purpurea (purple foxglove)

Summer

All replicates:

	Aromatic unknown 1	1.94 ± 0.61
Two replicates:		
	Benzoic acid	1.37 ± 0.55
	Chlorogenic acid 1	0.51 ± 0.00

Exclusive NI:
24, 52, 58, 61, 66, 76, 105, 117

Winter

All replicates:

	Benzoic acid	**2.79 ± 1.01**
One replicate:		
	Chlorogenic acid 1	0.68
	Chlorogenic acid 2	1.38
	Quercetin glycoside	0.63

Exclusive NI:
114

Begonia sutherlandii (Sutherland's begonia)

Summer

All replicates:

	Benzoic acid	**4.73 ± 1.55**
	Aromatic unknown 1	**12.9 ± 0.5**

Exclusive NI:
7

Winter

All replicates:		
	Benzoic acid	**7.42 ± 2.12**
One replicate:		
	Quercetin glycoside	1.26 ± 0.00
Exclusive NI: 16, 22, 30, 35, 70		

4. Discussion

4.1. Root exudate diversity (Experiment 1)

The GC–MS analyses detected several metabolites in the root exudates of more or less all accessed plant species, but not always in every replicate and in highly variable quantities. These including organic acids, amino acids, sugars, sugar alcohols, and one aromatic acid, benzoic acid (Table 7). Apart from benzoic acid, all are mentioned in literature summaries of root exudate components (UREN 2000, BERTIN et al. 2003, UREN 2007). Nevertheless, several reports suggest that benzoic acid also constitutes a widely occurring component of plant root exudates of herbaceous and woody plants (TANG & YOUNG 1982, YU and MATSUI 1994, DE LA FUENTE et al. 2007, TUASON and AROCENA 2009, LANUE et al. 2010).

Table 7: Common root exudate metabolites detected by GC–MS

Organic acids:	Malonic acid
	Succinic acid
	Malic acid
Amino acids:	Isoleucine
	Alanine
	Threonine
	Pyroglutamic acid (all except Fabaceae)
Sugar alcohols:	*myo*-Inositol
Sugars	Fructose
	Glucose
	Galactose
Aromatic compounds	Benzoic acid

A principle component analysis revealed a pronounced clustering of more or less all families except *Cucurbitaceae* and *Solanaceae*. The variation itself, however, was rather low (r^2 = 0.14 for principal component 1 and principal component 2; see Figure 1), reflecting that several of the major components of the root exudates were identical. Tomato, potato, pepper and cucumber, all of them exuded the amino acids isoleucine and pyroglutamic acid, the latter being an unusual amino acid that is regarded as a cyclisation product of glutamic acid (ABRAHAM & PODELL 1982). Furthermore, organic acids, malic acid, the sugar alcohol *myo*-inositol and the monosaccharide fructose were detected. The obtained results agree with previous studies of tomato root exudates (KRAVCHENKO et al. 2003, KAMILOVA et al. 2006a,b), as well as with those reported from pepper (KAMILOVA et al. 2006a) and cucumber (KAMILOVA et al.

2006a). Pea differed by exuding the amino acid homoserine, succinic acid and the sugar alcohol erythritol, and bean by exuding aspartic acid. The amino acids homoserine and aspartic acid have been reported as typical legume root exudate components (PATE 1962). Erythritol is a sugar alcohol that is mainly known from fruits and fermented foods. It was, however, reported recently to occur in the root exudates of the *Poaceae Lolium perenne* (CLAYTON et al. 2008) —concordantly, in this study, it was also detected in the barley root exudate. In contrast to the investigated *Solanaceae* and *Cucurbitaceae*, pyroglutamic acid was absent in the investigated legumes. In the barley root exudate, besides of the already mentioned erythritol, the amino acids leucine, isoleucine, and here again pyroglutamic acid, as well as fructose were detected as prevalent analytes. One existing study on barley root exudates congruently reports amino and organic acids (FAN et al. 1997), only instead of pyroglutamic acid glutamic acid is listed. White mustard exuded gulonic acid, a hydrolysis product of oxidized ascorbic acid, and galactose besides the common exudate components isoleucine, pyroglutamic acid and fructose. The carrot root exudate then contained as additional major component aspartic acid, similarly as the pea root exudate.

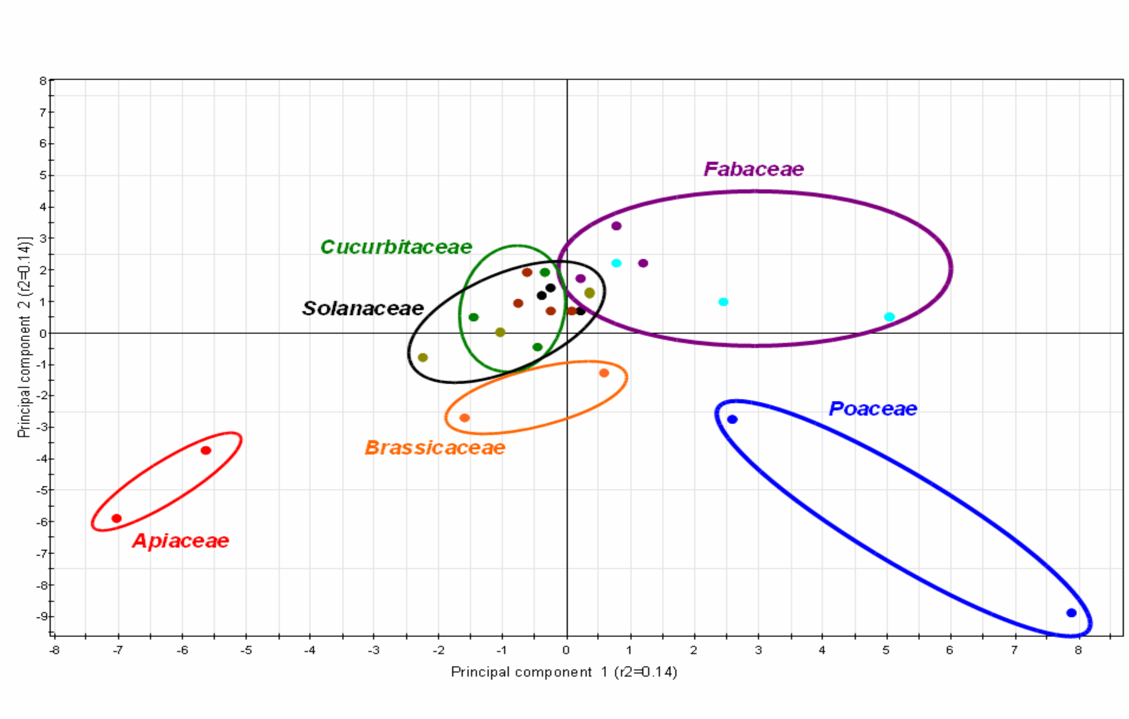

Figure 1: GC–MS-detected root metabolites (PCA) ● *Hordeum vulgare* ● *Daucus carota* ● *Sinapis alba* ● *Cucumis sativus* ● *Capsicum anuum* ● *Solanum lycopersicum* ● *Solanum tuberosum* ● *Phaseolus vulgaris* ● *Pisum sativum.*

The results from the GC–MS analyses reflected themselves in the PCA clusters of the HPLC–UV analyses (figure 2). Again no separate clusters distinguished *Solanaceae* and *Cucurbitaceae*; tryptophane, benzoic acid and various aromatic acids were detected. Pea differed by exuding pisatin, an isoflavone that was to be expected (NOVAK et al. 2004); by contrast, the bean exudate contained no detectable isoflavonoids. Carrot differed by accumulating ferulic acid and phenylacetic acid, besides several non-identified compounds. Ferulic acid has been described as root exudate constitutent of *Arabidopsis thaliana* (WALKER et al. 2003) and rice (SEAL et al. 2004) and most probably represents a widespread root exudate component. Phenylacetic acid was detected in hydroponic cultures of *Lactuca sativa* (LEE et al. 2006) and as root exudate component barley (LANOUE et al. 2010). Barley and white mustard differed by accumulating notable amounts of tyrosine besides tryptophane; occurrences of this amino acid have been reported for *Brassicaceae* (CAO et al. 1997) and *Poaceae* (MURATOVA et al. 2009). Of all plant species investigated, white mustard was the only one that exuded flavonoids, specifically kaempferol glycosides, which are known to occur in *Arabidopsis* root exudates (BADRI et al. 2008).

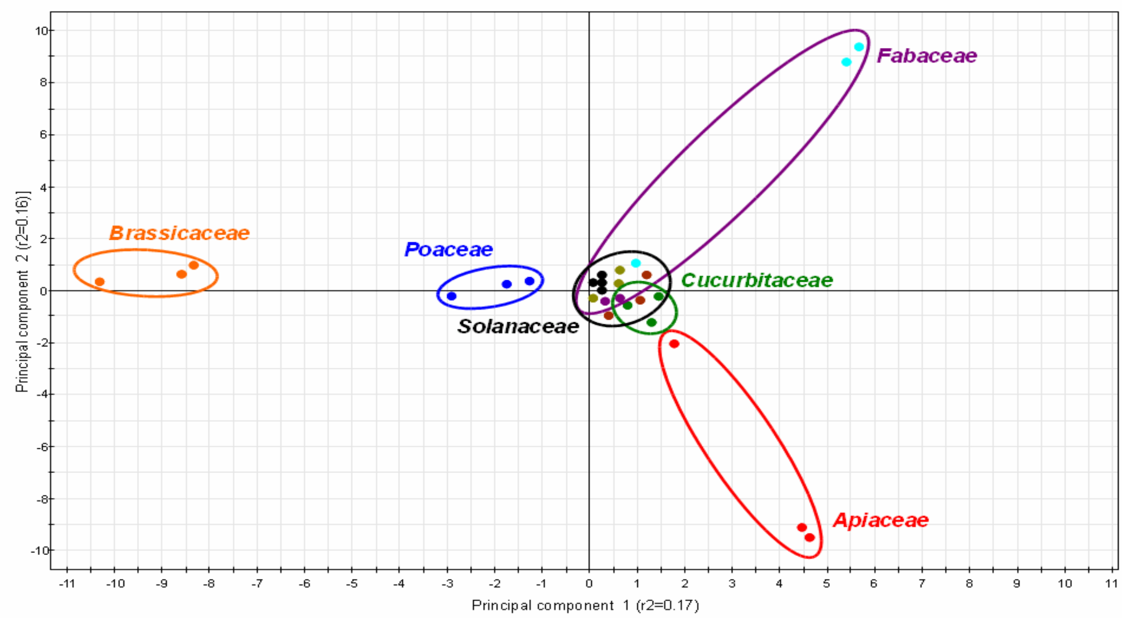

Figure 2: HPLC-UV-detected root metabolites (PCA) ● *Hordeum vulgare* ● *Daucus carota* ● *Sinapis alba* ● *Cucumis sativus* ● *Capsicum anuum* ● *Solanum lycopersicum* ● *Solanum tuberosum* ● *Phaseolus vulgaris* ● *Pisum sativum.*

4.2. Effects of mycorrhization and phosphorus supply (Experiment 2)

Barley, carrot, cucumber and tomato plants were infected with the arbuscular mycorrhizal fungus *Glomus mossae* and, concomitantly, a portion of the control plants were subjected to additional phosphorous treatments to determine if any changes in the root exudate metabolites were caused be increased phosphorus uptake by the plant and not by the colonization of the mycorrhizal fungus. The principle component analysis only indicated notable changes in the root exudate patterns of mycorrhized carrots. The concurs with the high degree of root colonization of *G. mossae* (71 %) in the treated carrot roots. By contrast, control and +P treatments clustered together (see Figure 3).

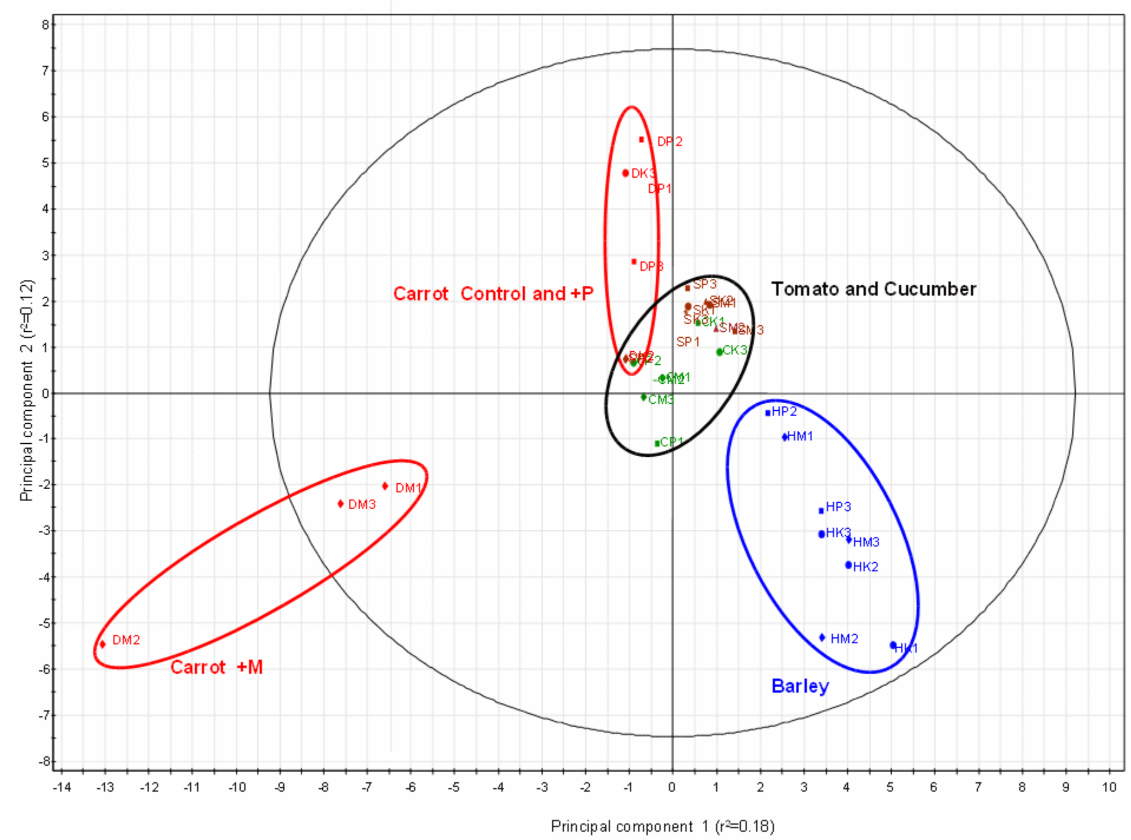

Figure 3: GC–MS detected metabolites (PCA) of treatments (●, control; ■, +P; ♦, +M [*Glomus mossae*]) on barley, carrot, cucumber and tomato.

Metabolites that contributed to the separate clustering of the +M treated carrot root exudate included *myo*-inositol, a sugar alcohol, which increased more or less 4 to 5-times compared to the control and +P treatments. Furthermore, maltotriose, only detectable in traces in the +P treatment increased 10-fold as third most-abundant component in the +M root exudate. Similarly, but less pronounced, this also applies to mannitol, a further sugar alcohol as well as NI3 and NI11, two non-identified compounds. Concomitantly, the levels of the sugars fructose, glucose, and galactose dropped dramatically.

The specific effect of mycorrhizal colonization reflected itself in the fact that the failed colonisation of the barley plants resulted in one cluster of the treatments. In this experiment, the barley plants also exuded notable amounts of leucine and isoleucine, but in contrast to the first experiment, the sugar alcohol erythritol was not detected. Also, pyroglutamic acid was replaced by γ-aminobutyric acid (see 4.1). Nevertheless, these characteristics contributed to a more or less separate clustering of the barley plants in the PCA (see Figure 3). The treatments of cucumber and tomato, despite better colonization rates than barley, but still worse than carrot, did not cause specific effects on root exudate quality. Similary as in the first experiment (see 4.1), cucumber and tomato root exudates clustered together. In this experiment, succinic acid was more prevalent in the root exudates of all plant species. Again, fructose and *myo*-inositol were prominent components of the root exudates. Isoleucine and pyroglutamic acid were less prominent, only barley exuded notable amounts of amino acids. The absence of pyroglutamic acid and more pronounced presence of γ-aminobutyric acid may be explained by the fact that both have a common precursor, glutamic acid. Whereas pyroglutamic acid is regarded as an artificial cyclisation product of glutamic acid (ABRAHAM & PODELL 1982), γ-aminobutyric acid is a stress-induced metabolite and signal compound in plant tissues and usually leads to elevated levels of succinic acid that are aimed at fuelling the respiratory chain (BOUCHÉ & FROMM 2004).

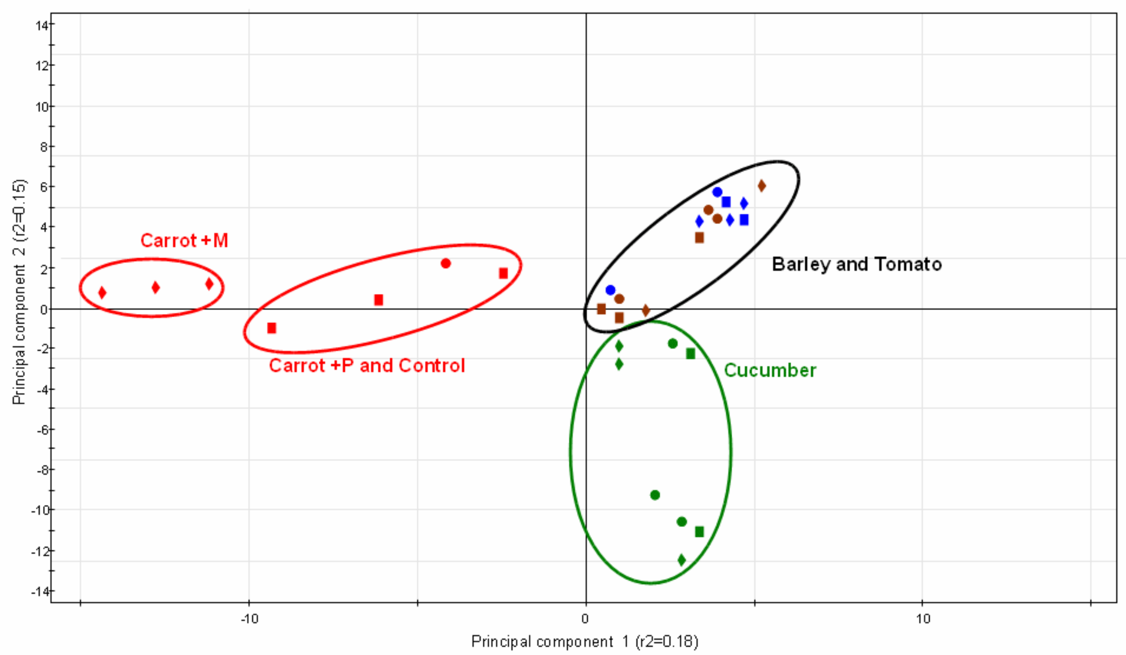

Figure 4: HPLC-UV detected metabolites (PCA) of treatments (●, control; ■, +P; ♦, +M [*Glomus mossae*]) on barley, carrot, cucumber and tomato.

The HPLC analyses somehow matched with the results from GC analyses as benzoic acid represent the most prominent compound in most of the analysed samples. In a previous experiment where the same plant species were used this was not the case, concurring with γ-aminobutyric acid from the GC analyses. Again, the exception was carrot, where, most probably due to the high degree of mycorrhization, 27 unique peaks emerged in the colonized roots compared to 9 in the control and 13 in the +P treated plants, indicating a fundamental reprogramming of the metabolism. Generally, carrot root exudates contained more metabolites than barley, cucumber and tomato. Contrary to the GC analyses , where the tomato and cucumber treatments clustered together, the HPLC analyses afforded a single cluster for barley and tomato. Cucumber differed from those two by accumulating more cinnamic acid derivatives, especially sinapinic and syringic acid. Barley, cucumber and tomato resembled each other in terms of HPLC–UV detectable metabolites because all exuded the amino acid phenylalanine in notable amounts, which was not detectable in carrot. Similarly as benzoic acid, phenylalanine was more prominent in the second than in the first experiment series.

4.3. Benzoic acid seasonal dynamics (Experiment 3)

In this experiment, benzoic acid concentrations were the main focus (thus, the restriction to HPLC). According to expectations, concentrations differed between seasons, but not congruently as originally predicted (see Figure 5).

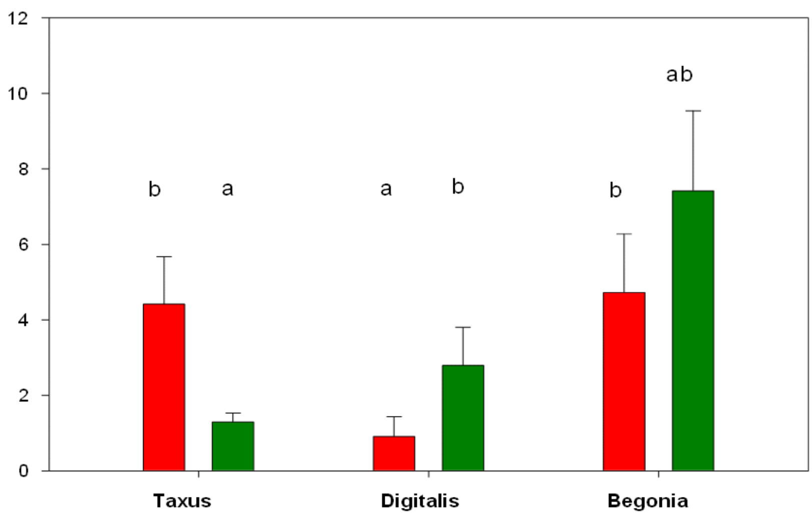

Figure 5. Benzoic acid concentrations (5 peak area) in summer (red bar) and winter (green bar); mean + SD, ANOVA with Duncan's multiple range test (n=3).

In the woody *Taxus*, benzoic acid concentrations were lower in winter than in summer. In the two herbaceous species, benzoic acid concentrations were higher in winter than in summer as originally expected. Only investigations including more accessions of herbaceous and woody plants will show if this phenomenon represents a robust characteristic.

Despite these differences, however, late spring and winter accessions of these three unrelated plant species formed distinctive clusters for the specific season (see Figure 6). This was caused by numerous season-specific analytes that occurred in at least two of the three species (see 3.3).

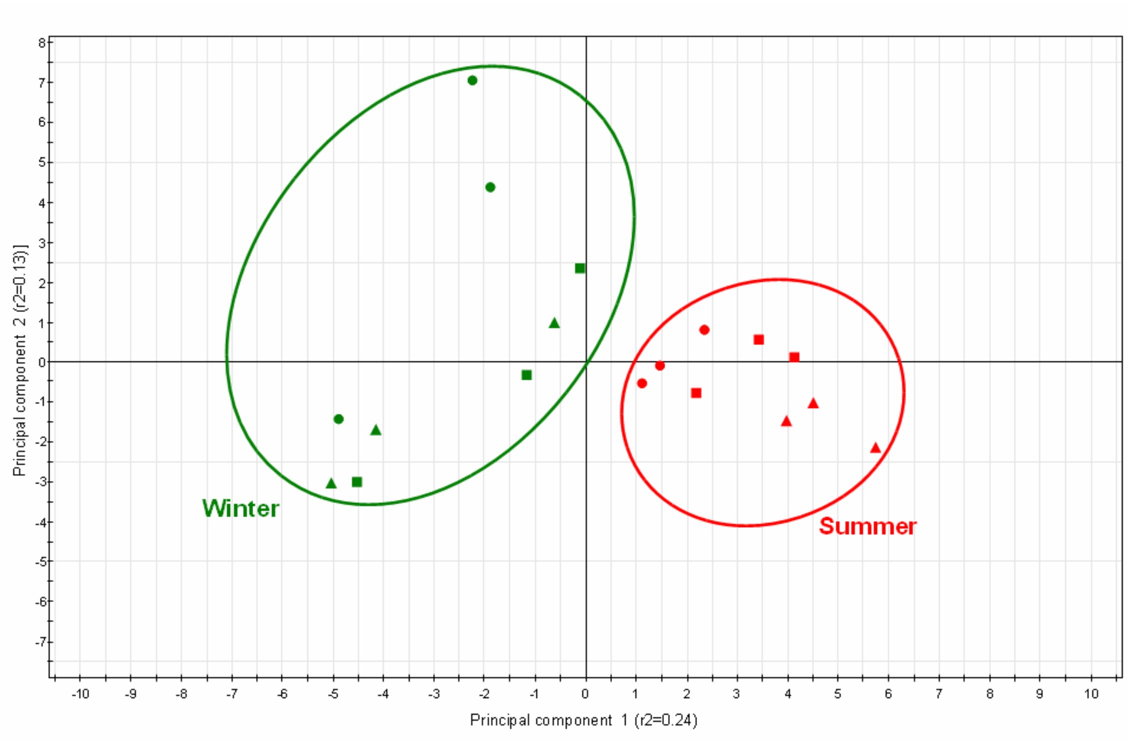

Figure 6. PCA of seasonal root exudate accessions, wintrer green and summer red (●, *Begonia sutherlandii*; ■, *Digitalis purpurea*; ▲, *Taxus baccata*).

4.4. Conclusions

To a certain extent, the composition of root exudates reflects plant phylogenetic relationships, but environmental conditions and biotic interactions may cause fundamental changes in their composition. Separation of the crude exudate into a hydrophilic fraction (analyzed by GC–MS) and a lipophilic fraction (analyzed by HPLC–UV) represents a practical approach to obtain information about the quantity and quality of root-exuded metabolites. Various collection methods exist and none is perfect. The one applied here, washing soil-grown plants in distilled water (after careful removal of the soil), proved as useful to a certain extent. The obtained results concur more or less with previous investigations if some are available.

One limitation, however, is that our collection method, nor any other applied for this purpose, quantifies the actual metabolite amounts that are exuded during the 4 h collection period, but what is recoverable from the root mucilage by aqueous extraction. This explains the high variability between the replicates and also recommends further studies to explore the relations between metabolite exudation and root mucilage formation. Different polymerisation rates of the exuded metabolites may explain the fact that, for a number of accessions, chromatographic analysis was not possible despite accessible quantitative yields.

Plant growing conditions also may exert a pronounced effect on the root exudate qualtity and quantity. Notable differences in quality and quantity of the same plant species between the two experiment series were detected. In the second experiment, in which the effect of mycorrhization of root exudate quality was explored, succinic acid instead of malic acid, γ-aminobutyric acid instead of pyroglutamic (or originally glutamic acid), and more pronounced amounts of benzoic acid were detectable compared to the first experiment series that focussed on interspecific variation. The mentioned metabolites belong to various classes and all can be attributed to stress affecting respiration, in particular the citric acid cycle (succinic and γ-aminobutyric acid) (BOUCHÉ & FROMM 2004). Nevertheless, if the mycorrhizal fungal succeeded in colonising the plant thoroughly, as this was in the case of carrot, a pronounced reprogramming in the metabolisms became evident. Further studies are required to determine if higher colonisation rates correlate with more pronounced exudate metabolite profiles. In this study, higher additional supply of phosphorus did not affect metabolite patterns from those plants that received lower supplies. Moreover; contrary to literature reports (DINKELAKER et al. 1997, BADRI et al. 2009), lower phosphorus supplied plants did not exude more phenolic compounds than better supplied. Perhaps the lower supply was not deficient enough. The phenomenon that phosphorous-deficient plants exude larger amounts of organic acids is considered currently as variable (BADRI et al. 2009).

One specific aspect that arose during the investigation if seasonal variation of benzoic acid concentration represents a robust characteristic of root exudates. All three randomly chosen plant species differed in their benzoic acid concentrations present in winter and late spring, but only one species (*Taxus*) exuded relatively more benzoic acid in winter than in late spring. The other two species showed the opposite effect. Interestingly, the root exudate profiles (HPLC–UV) of these three randomly selected species clustered according to the season. This suggest that there may exist season-specific metabolite exudation patterns that even are wide-spread among plant species.

All obtained results have to be tested with more species and accessions to allow robust predictions of the discussed phenomena. However, the so far obtained results in concert with published studies, strongly recommend further research in root exudate dynamics.

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6. Appendix

6.1. MS spectra

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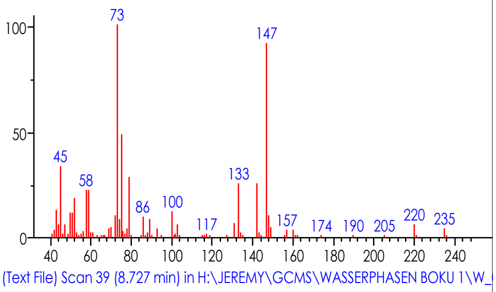
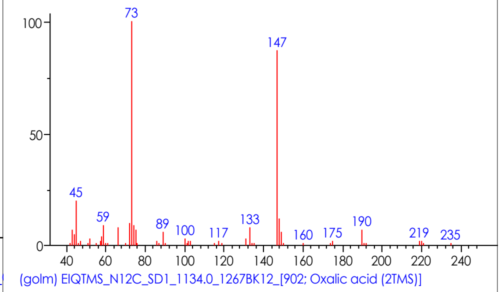
Oxalic acid</td><td></td><td></td></tr>
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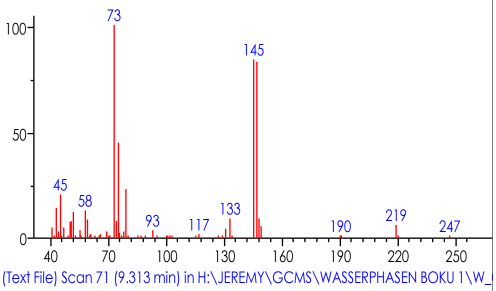
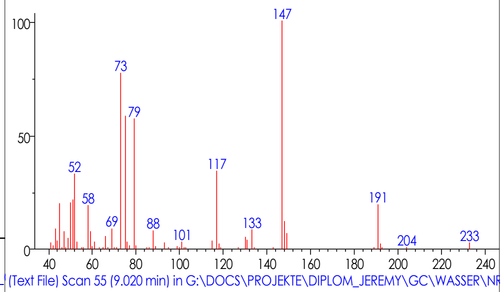
Lactic acid</td><td></td><td></td></tr>
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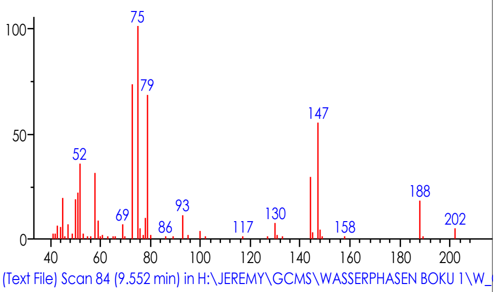
NI 1</td><td></td><td></td></tr>
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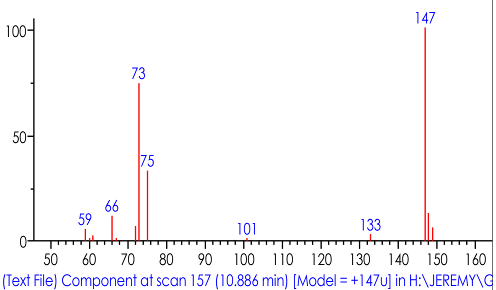
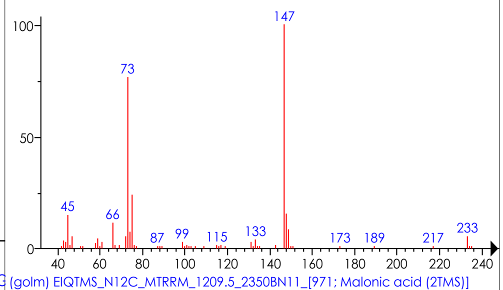
acid</td><td></td><td></td></tr>
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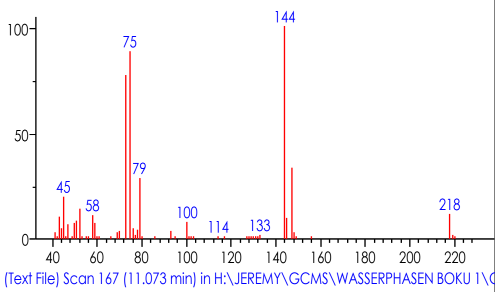
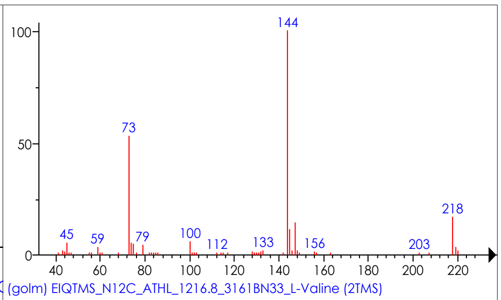
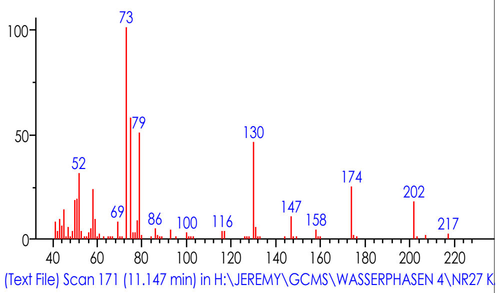
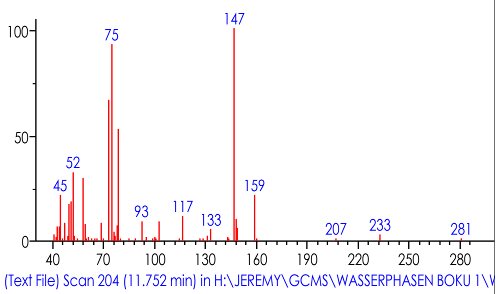
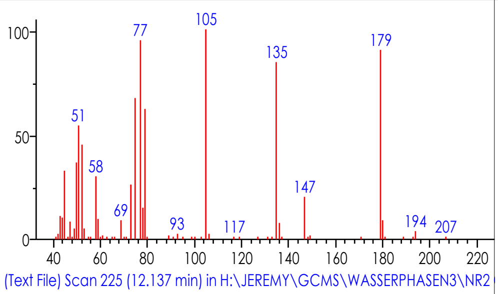
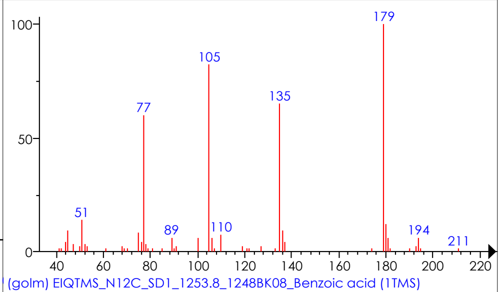
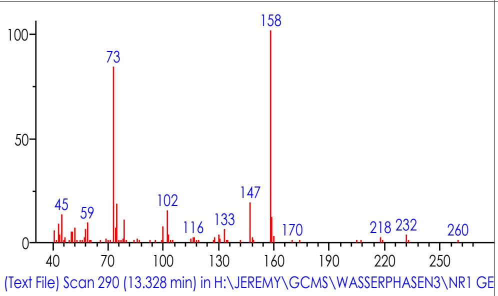
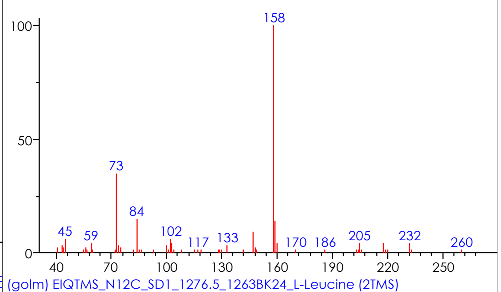
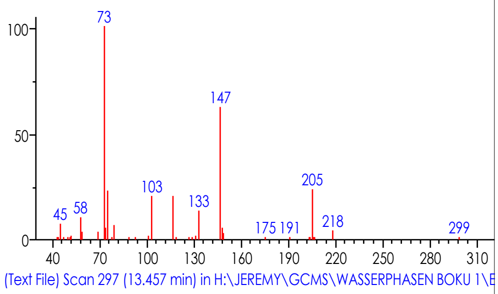
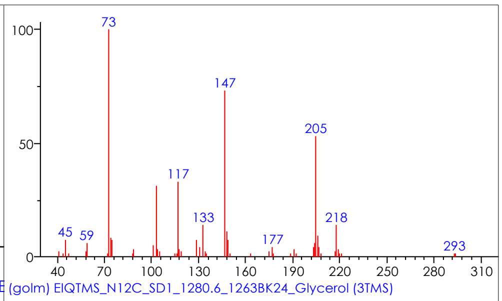
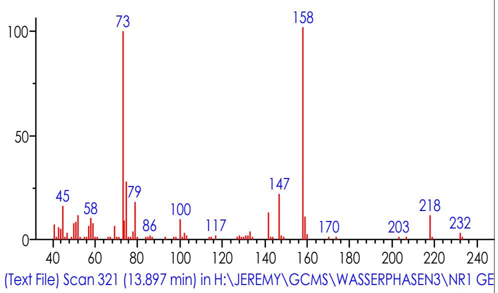
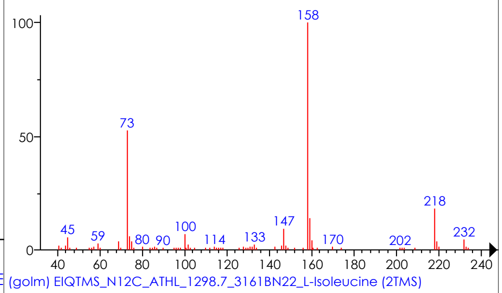
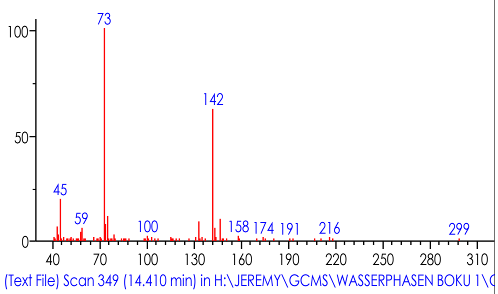
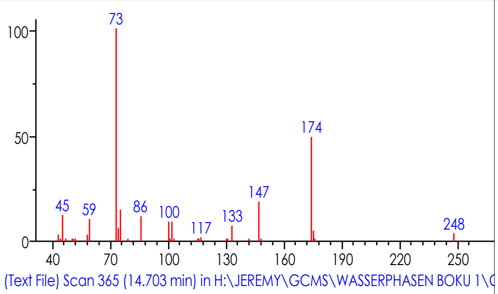
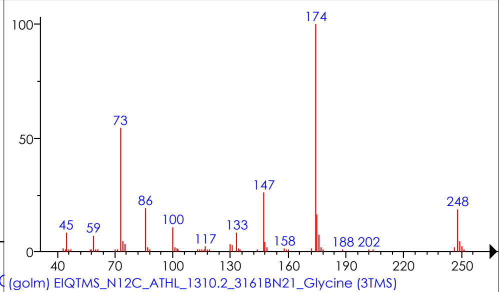
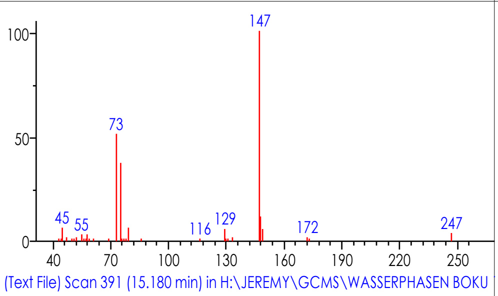
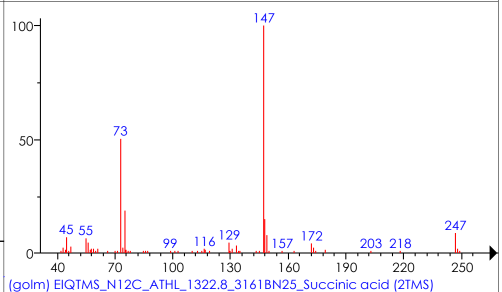
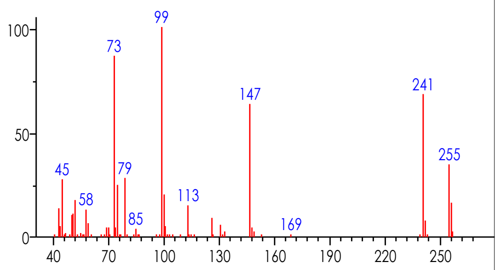
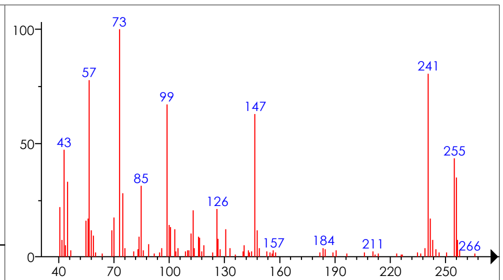
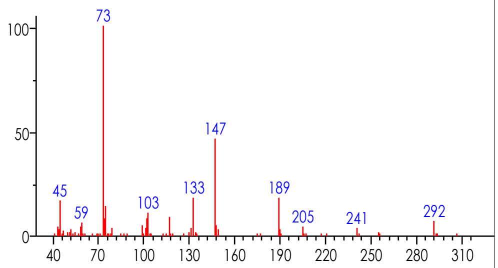
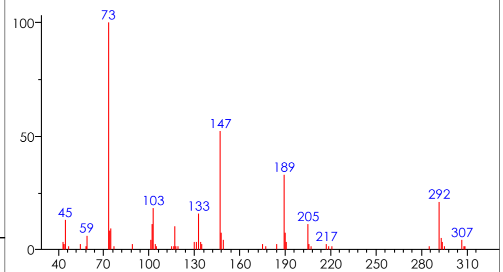
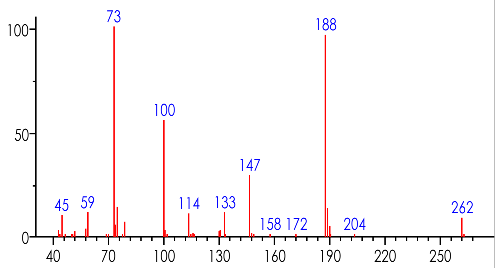
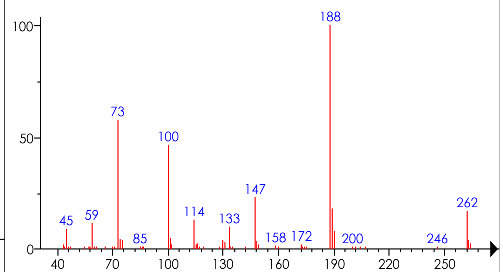
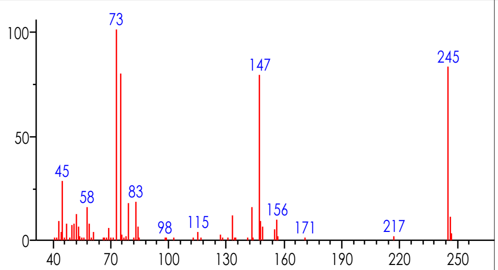
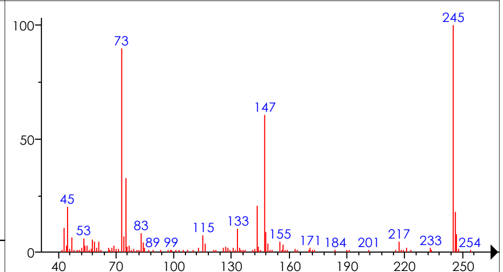
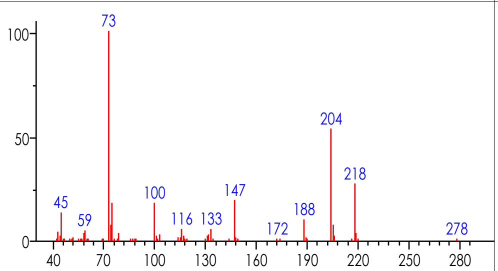
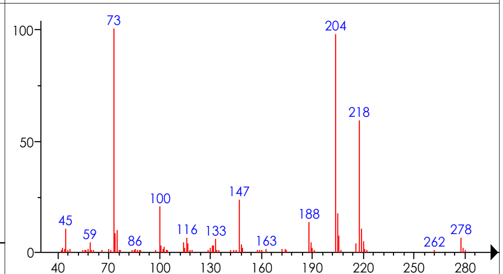
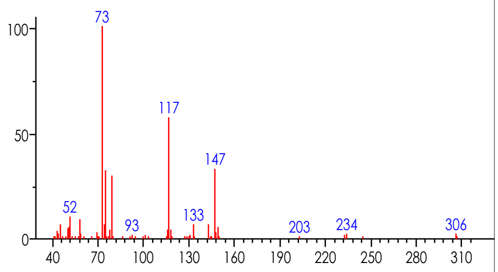
11.07 **Valine**		
11.17 **NI 2**		
11.75 **NI 3**		
12.17 **Benzoic acid**		
13.27 **Leucine**		

13.45 Glycerol		
13.89 Isoleucine		
14.41 NI 4		
14.70 Glycine		
15.18 Succinic acid		

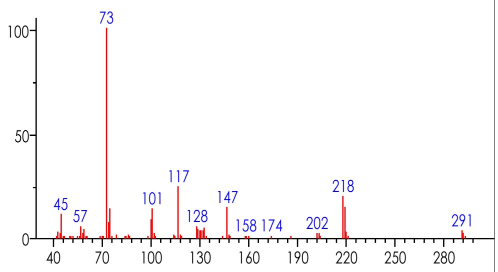
15.80 **Uracil**	 (Text File) Scan 425 (15.803 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR1	 (golm) EIQTMS_N12C_ATHL_1347.0_3161BN34_Uracil (2TMS)
15.88 **Glyceric acid**	 (Text File) Scan 429 (15.877 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU	 (golm) EIQTMS_N12C_SD1_1338.9_1267BK12_Glyceric acid (3TMS)
16.46 **Alanine**	 (Text File) Scan 461 (16.463 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU	 (golm) EIQTMS_N12C_ATHL_1362.7_3161BN21_L-Alanine (3TMS)
16.66 **Fumaric acid**	 (Text File) Scan 466 (16.556 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR3	 (golm) EIQTMS_N12C_OARPLAS_1360.4_3350DF06_Fumaric acid (2TMS)
16.98 **Serine**	 (Text File) Scan 488 (16.958 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU	 (golm) EIQTMS_N12C_ATHL_1367.4_3161BN33_L-Serine (3TMS)

17.32
NI 5

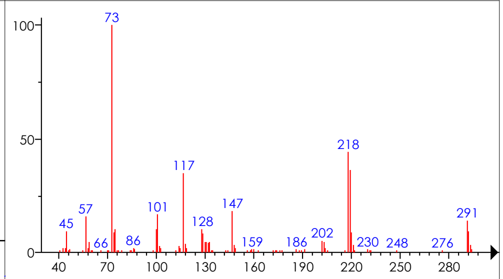


(Text File) Scan 508 (17.326 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 2\B

17.93
Threonine

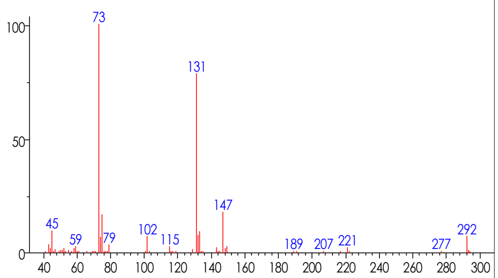


(Text File) Scan 547 (18.040 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU



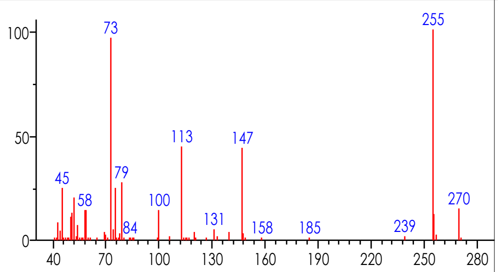
(golm) EIQTMS_N12C_ATHL_1392.0_3161BN20_L-Threonine (3TMS)

18.05
NI 6

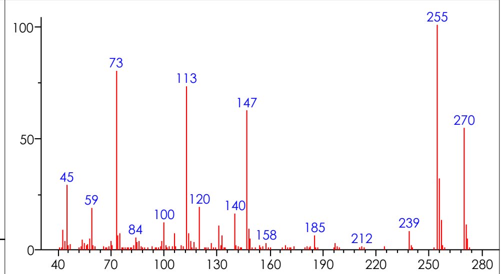


(Text File) Scan 546 (18.022 min) in G:\DOCS\PROJEKTE\DIPLOM_JEREMY\GC\WASSER\

18.22
Thymine

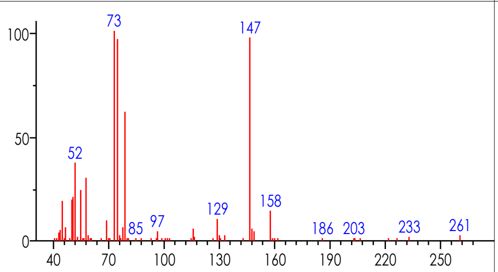


(Text File) Scan 558 (18.242 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR2

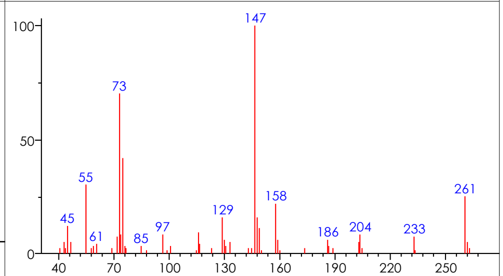


(golm) EIQTMS_N12C_SD1_1407.7_3209BF16_Thymine (2TMS)

18.71
Glutaric acid



(Text File) Scan 584 (18.718 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR2



(golm) EIQTMS_N12C_SD1_1413.4_1340AU21_Glutaric acid (2TMS)

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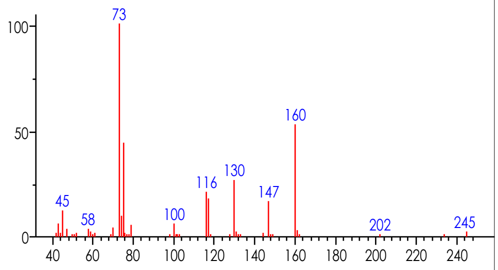
NI 7</td>
<td>

(Text File) Scan 645 (19.837 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\</td>
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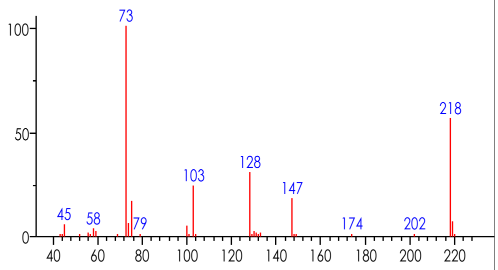
<td>20.52
Homoserine</td>
<td>

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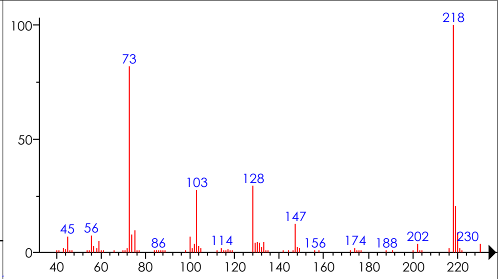
<td>

(golm) EIQTMS_N12C_SD2_1454.6_2114BV43_L-Homoserine (3TMS)</td>
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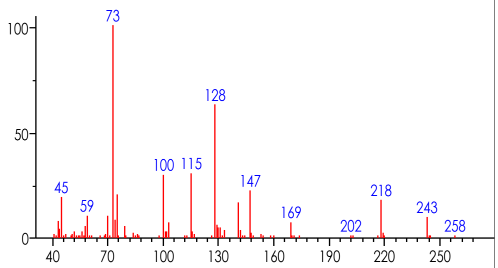
NI 8</td>
<td>

(Text File) Scan 688 (20.625 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\</td>
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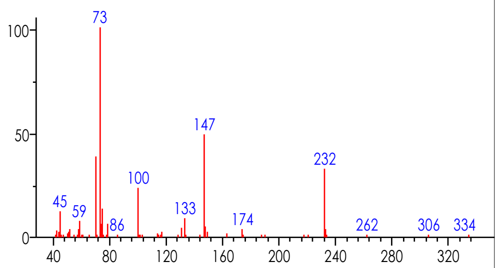
<td>20.97
Pyruvic acid</td>
<td>

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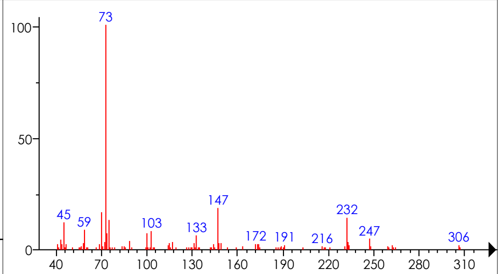
<td>

(golm) EIQTMS_N12C_CPEL_1473.8_2350BN57_[727; Pyruvic acid oxime (2TMS)]</td>
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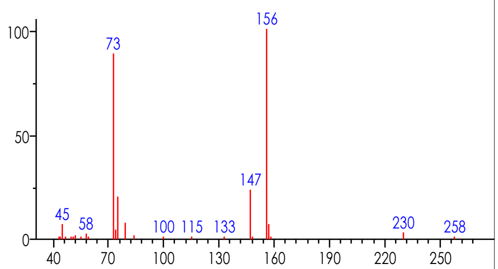
<td>21.21
Pyro-
glutamic
acid</td>
<td>

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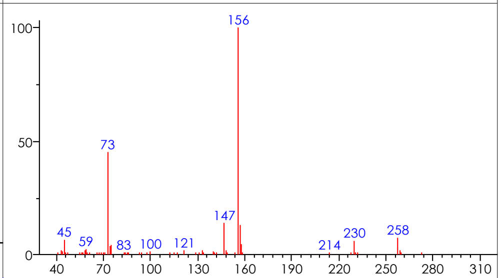
<td>

(golm) EIQTMS_N12C_LJARP_1527.6_2236BN20_Pyroglutamic acid (2TMS)</td>
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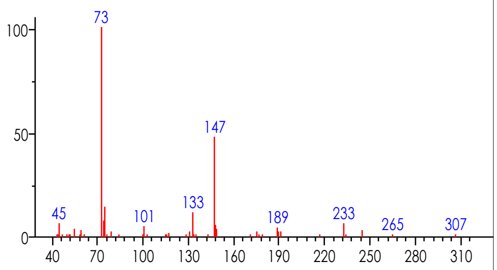
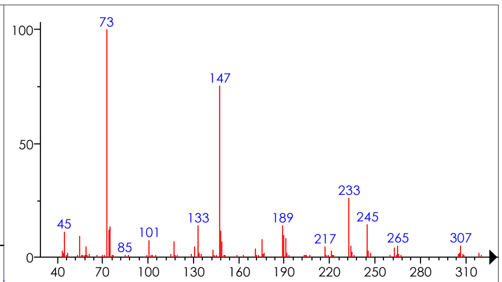
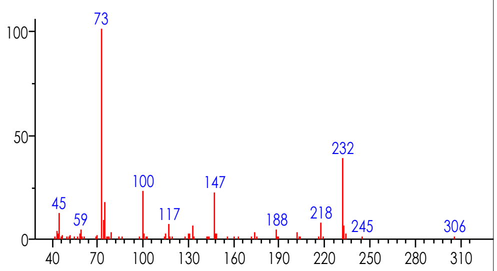
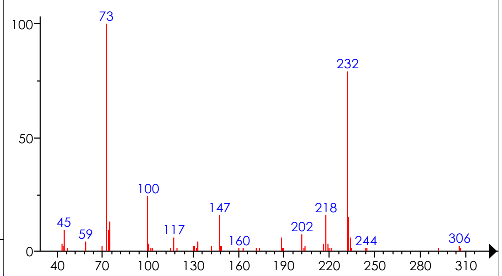
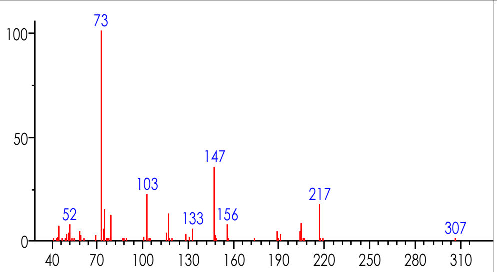
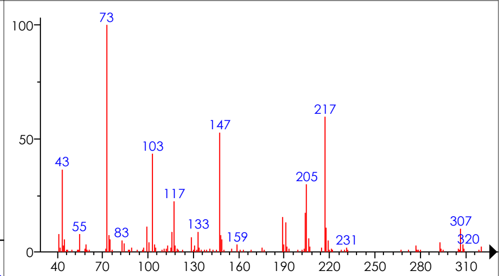
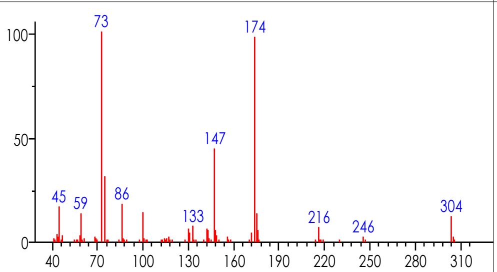
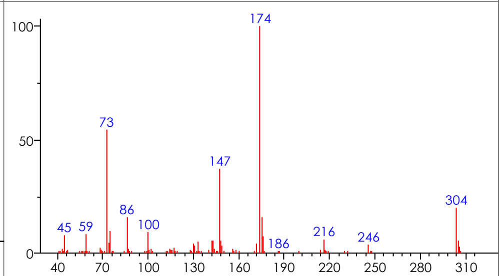
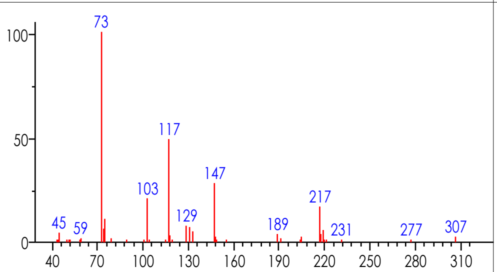
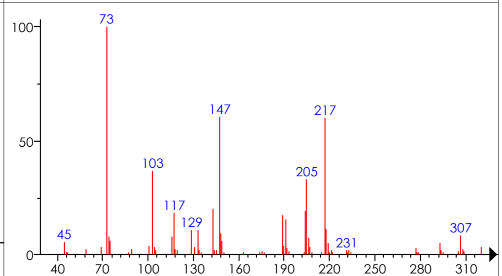
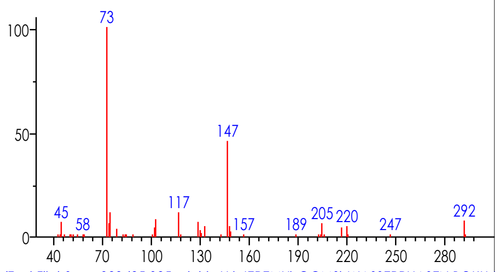
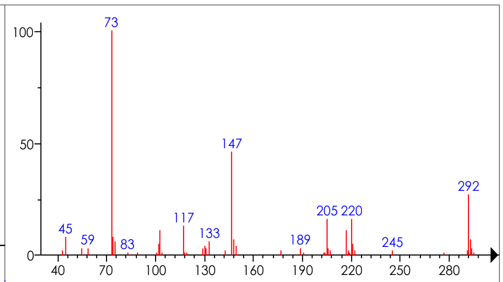
22.18 **Malic acid**	 (Text File) Scan 774 (22.202 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU	 (golm) EIQTMS_N12C_ATHL_1491.4_3161BN27_Malic acid (3TMS)
22.55 **Aspartic acid**	 (Text File) Scan 793 (22.550 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU	 (golm) EIQTMS_N12C_SD1_1525.1_1267BK12_L-Aspartic acid (3TMS)
22.80 **Threitol**	 (Text File) Scan 807 (22.807 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR2	 (golm) EIQTMS_N12C_LJALM_1501.9_2236BN40_Threitol (4TMS)
23.40 **4-Amino-butyric acid**	 (Text File) Component at scan 839 (23.390 min) [Model = +73u] in H:\JEREMY\	 (golm) EIQTMS_N12C_SD1_1531.1_3184BF15_4-Aminobutyric acid (3TMS)
23.74 **Erythritol**	 (Text File) Scan 860 (23.779 min) in H:\JEREMY\GCMS\WASSERPHASEN 5\NR3	 (golm) EIQTMS_N12C_LJALD_1510.1_2236BN30_Erythritol (4TMS)

24.38 Threonic acid

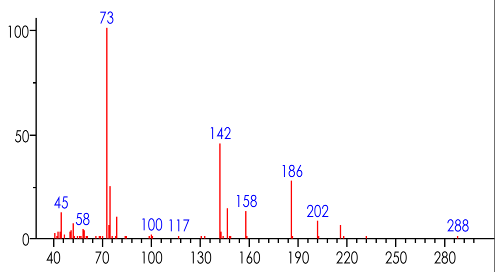


(Text File) Scan 928 (25.025 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU

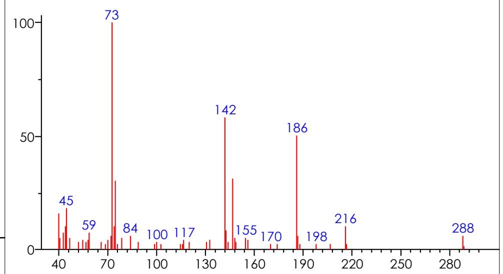


(golm) EIQTMS_N12C_SD1_1569.3_1267BK12_Threonic acid (4TMS)

25.23 Proline

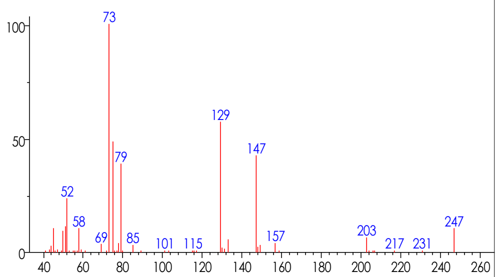


(Text File) Scan 938 (25.209 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR5



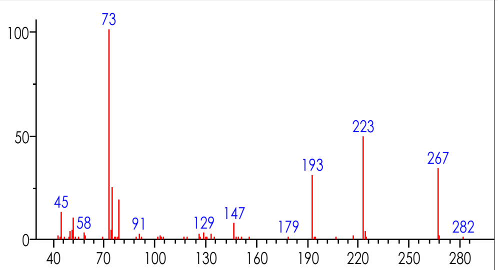
(golm) EIQTMS_N12C_NID1_1592.5_1325BK16_[612; Proline (2TMS)]

25.50 NI 9

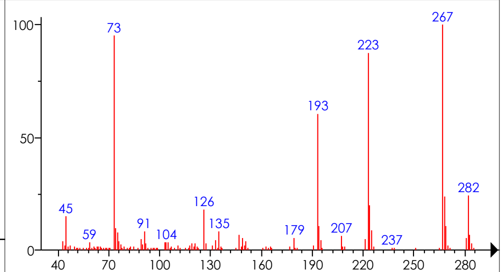


(Text File) Scan 944 (25.319 min) in G:\DOCS\PROJEKTE\DIPLOM_JEREMY\GC\WASSER\

26.85 4-Hydroxy-benzoic acid

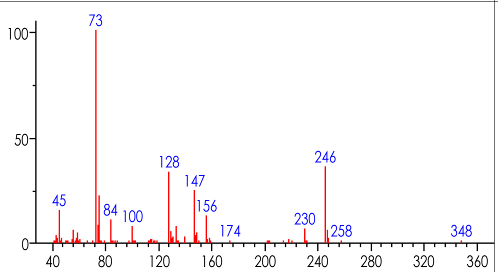


(Text File) Scan 1028 (26.859 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR2

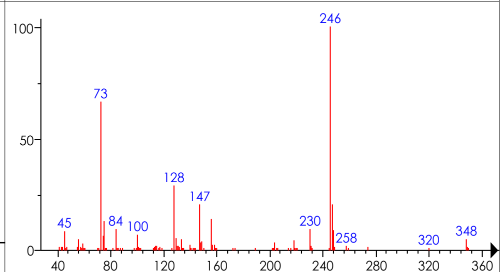


(golm) EIQTMS_N12C_SD1_1638.3_3035BB03_4-Hydroxybenzoic acid (2TMS)

27.30 Glutamic acid

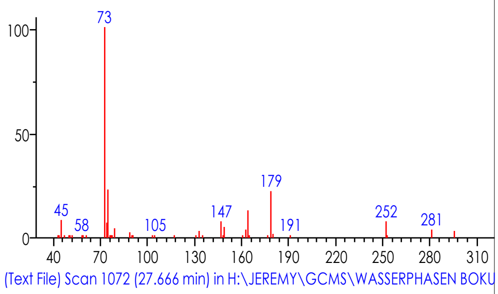


(Text File) Scan 1057 (27.391 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\

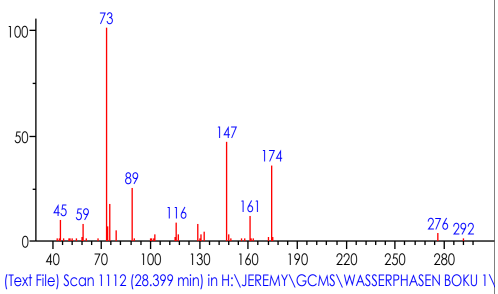


(golm) EIQTMS_N12C_LJALM_1632.2_2236BN40_L-Glutamic acid (3TMS)

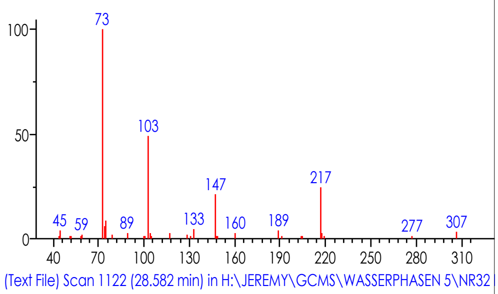
27.50 Phenylacetic acid

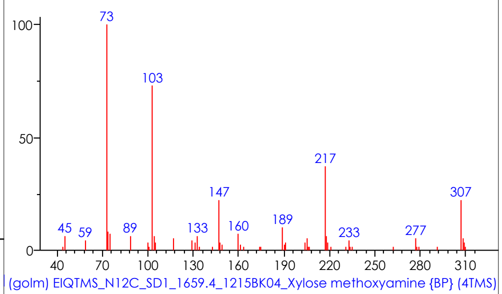


28.39 NI 10

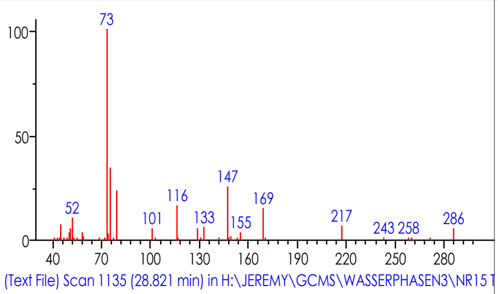


28.61 Xylose

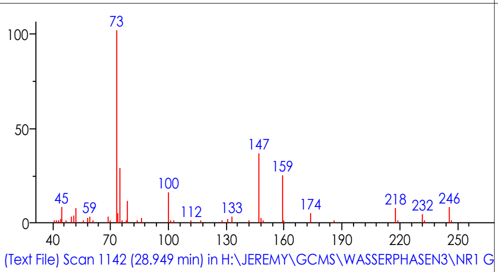


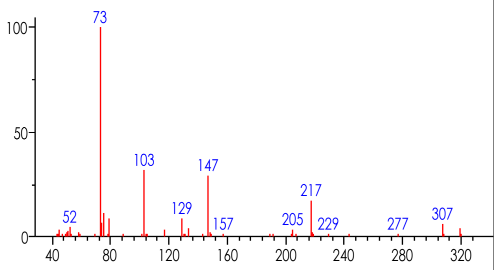
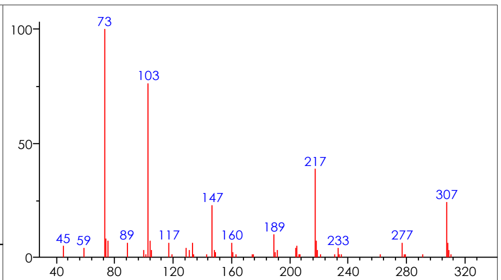
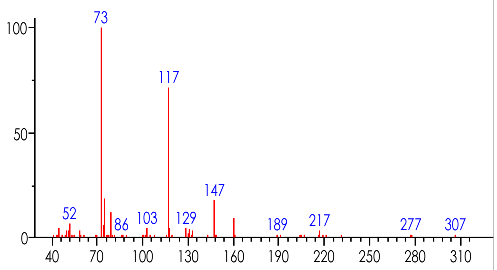
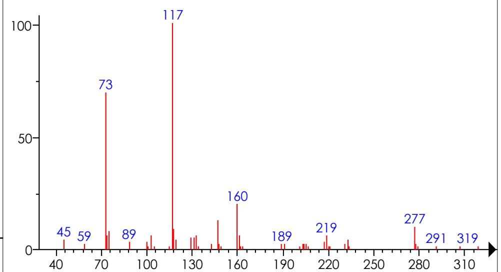
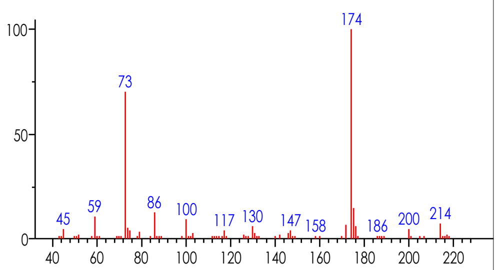
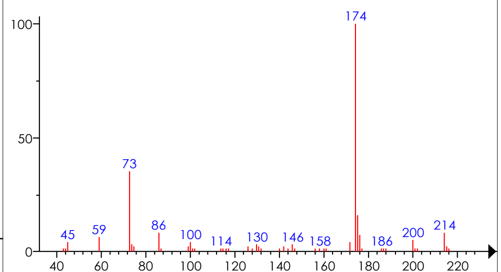
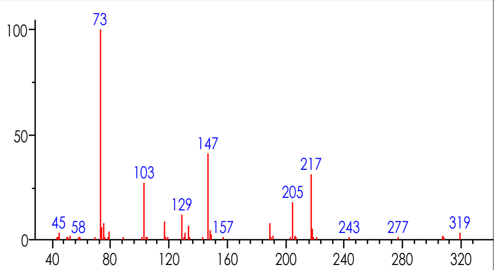
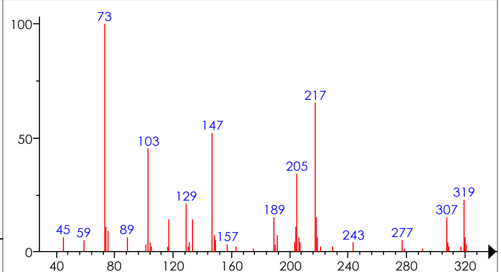
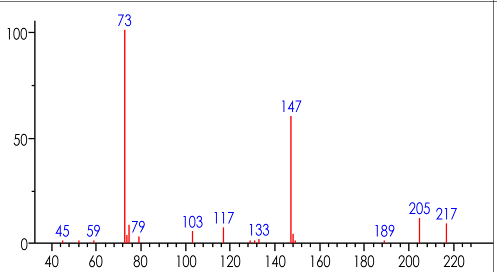


28.82 NI 11



28.94 NI 12



29.57 **Arabinose**	 (Text File) Scan 1176 (29.572 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR21	 (golm) EIQTMS_N12C_SD2_1673.8_1215BK02_Arabinose methoxyamine (4TMS
30.41 **Rhamnose**	 (Text File) Scan 1222 (30.416 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR30	 (golm) EIQTMS_N12C_SD1_1733.2_1215BK16_Rhamnose methoxyamine {BP} (4
30.45 **Putrescine**	 (Text File) Scan 1224 (30.452 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR14	 (golm) EIQTMS_N12C_SD1_1740.7_1334BV11_Putrescine (4TMS)
30.67 **Ribitol**	 (Text File) Scan 1233 (30.617 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR21	 (golm) EIQTMS_N12C_SD1_1735.2_1267BK12_Ribitol (5TMS)
31.14 **NI 13**	 (Text File) Scan 1262 (31.149 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	

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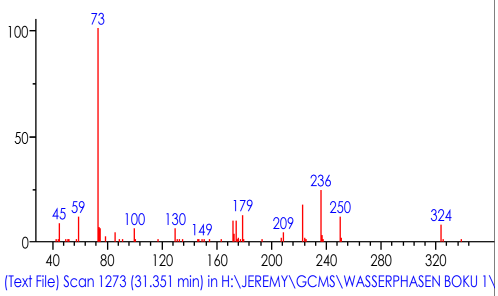
NI 14</td><td>
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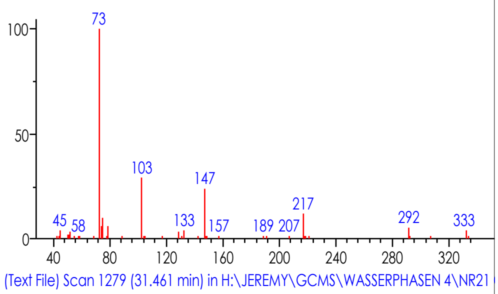
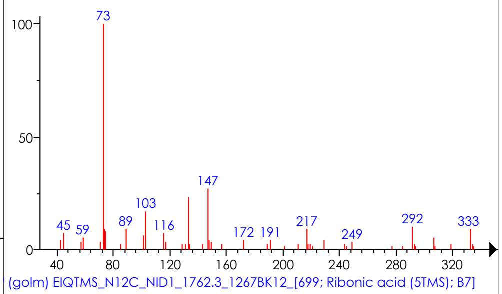
Ribonic acid</td><td>
</td><td>
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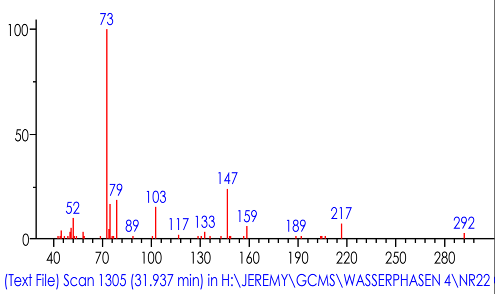
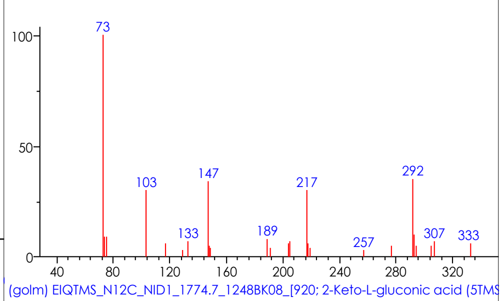
2-keto-l-gluconic acid</td><td>
</td><td>
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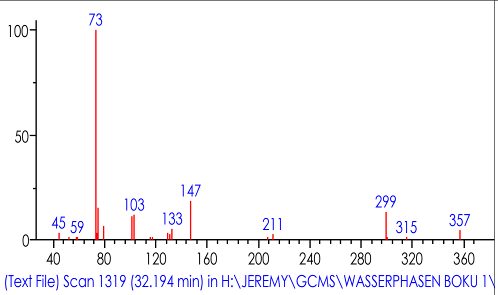
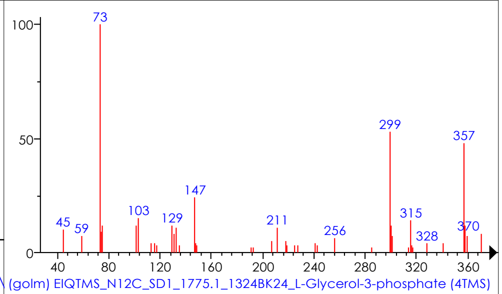
Glycerol-3-phosphate 1</td><td>
</td><td>
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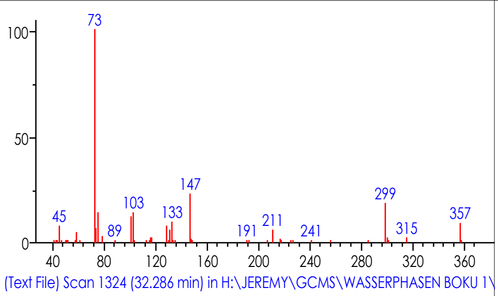
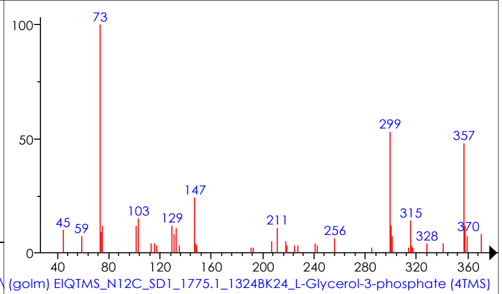
Glycerol-3-phosphate 2</td><td>
</td><td>
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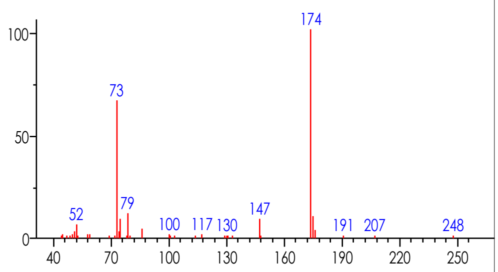
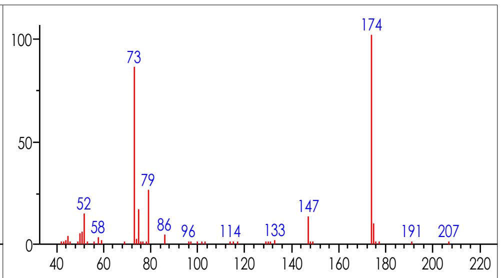
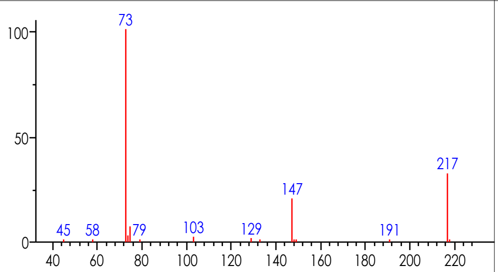
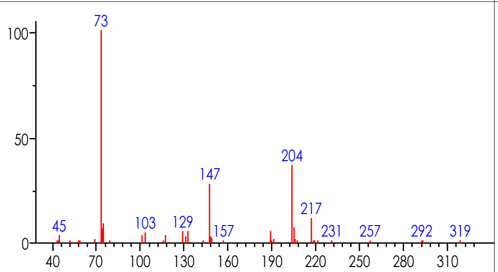
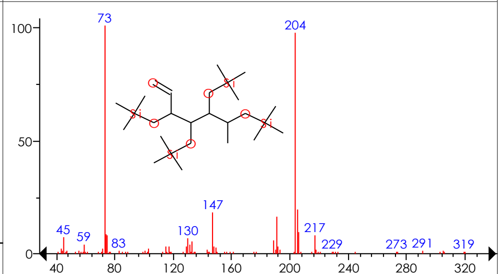
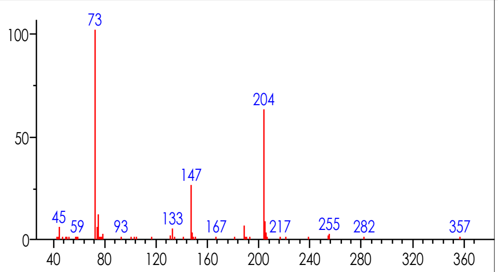
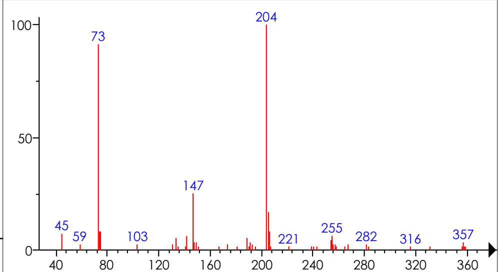
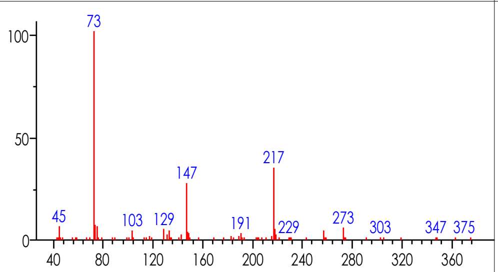
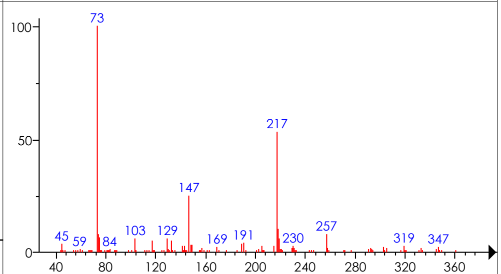
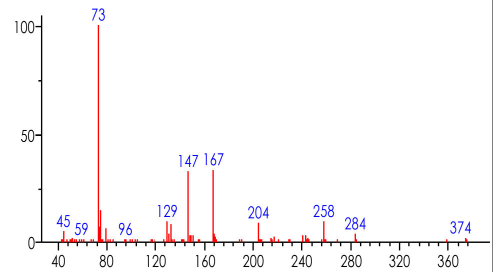
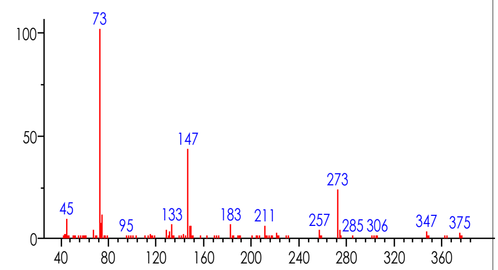
32.81 - Histamine	 (Text File) Scan 1354 (32.836 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR22	 (Text File) Scan 1353 (32.817 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR23
33.40 NI 16	 (Text File) Scan 1385 (33.404 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	
33.60 Mannose	 (Text File) Scan 1397 (33.624 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	 (replib) Mannose, 6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, L-
33.99 Shikimic acid	 (Text File) Scan 1417 (33.991 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR24 (	 (golm) EIQTMS_N12C_SD1_1822.2_1267BK12_Shikimic acid (4TMS)
34.10 Fructose	 (Text File) Scan 1422 (34.082 min) in H:\JEREMY\GCMS\WASSERPHASEN 5\NR32	 (golm) EIQTMS_N12C_ATHL_1809.7_3161BN26_[798; Fructose (5TMS)]

34.13 - NI 17

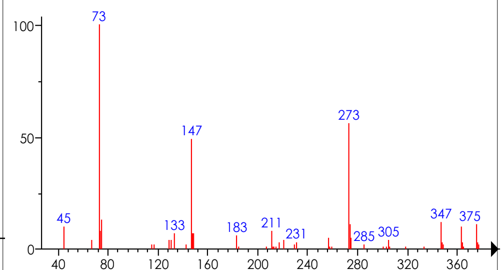


(Text File) Scan 1425 (34.137 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR3

34.30 Citric acid

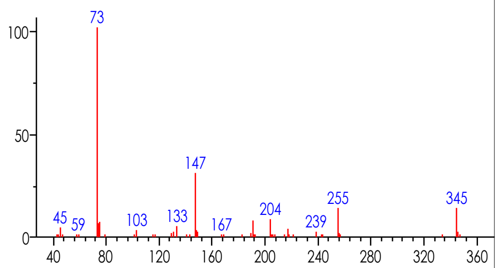


(Text File) Scan 1419 (34.027 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR2

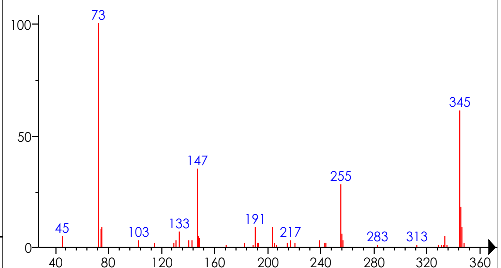


(golm) EIQTMS_N12C_SD1_1828.1_1267BK12_Citric acid (4TMS)

35.16 Quinic acid

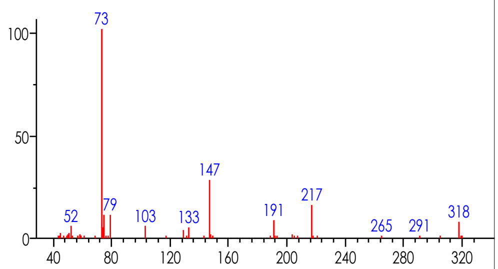


(Text File) Scan 1487 (35.274 min) in H:\JEREMY\GCMS\WASSERPHASEN 5\NR32

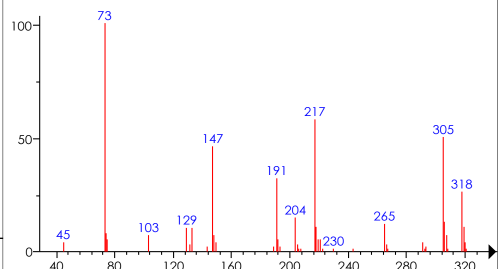


(golm) EIQTMS_N12C_SD1_1862.9_1267BK12_D(-)-Quinic acid (5TMS)

35.89 *scyllo*-Inositol

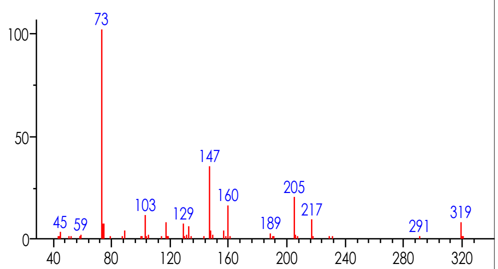


(Text File) Scan 1521 (35.897 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR29

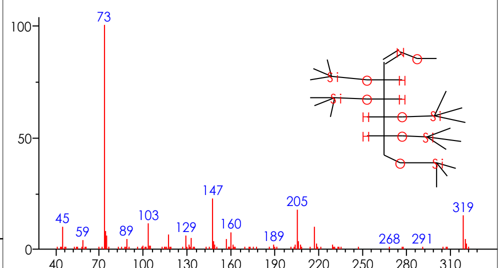


(golm) EIQTMS_N12C_SD1_2091.6_1267BK12_myo-Inositol (6TMS)

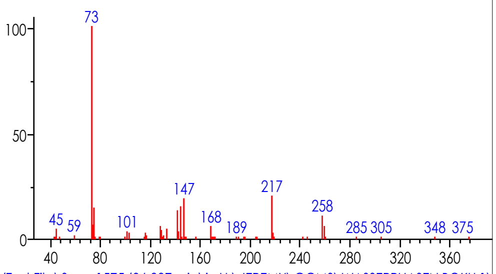
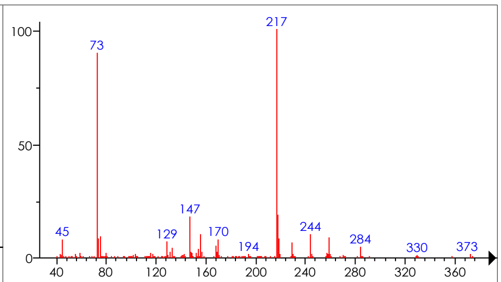
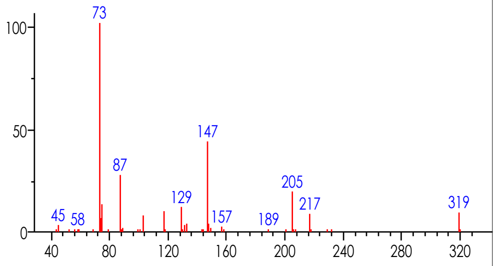
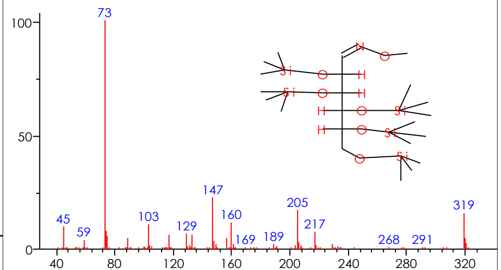
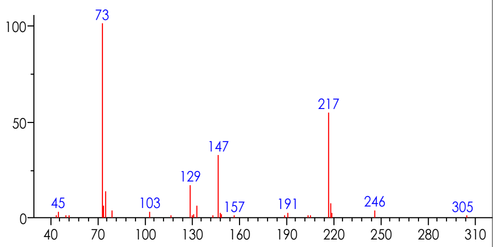
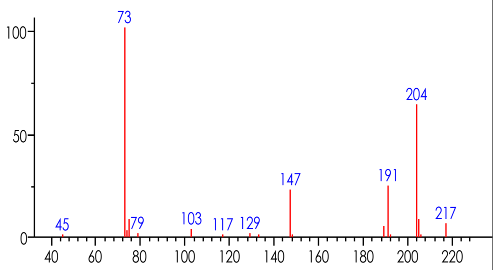
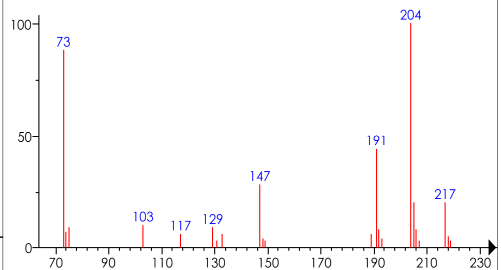
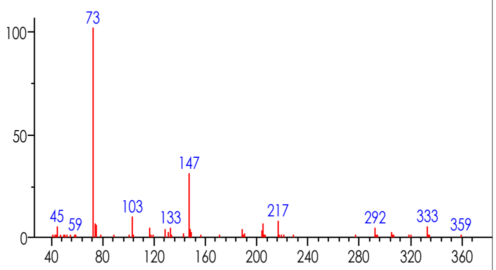
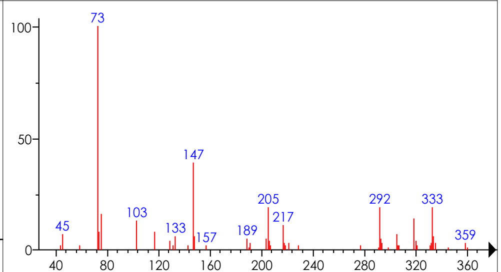
36.50 Galactose



(Text File) Scan 1554 (36.503 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\



(mainlib) d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-

36.88 **Calystegine**	 (Text File) Scan 1575 (36.887 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	 (golm) EIQTMS_N12C_SD1_1974.6_3233BF05_Calystegine B2 B4 (4TMS)
36.89 **Glucose**	 (Text File) Scan 1577 (36.924 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	 (mainlib) d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-
38.40 **NI 18**	 (Text File) Scan 1658 (38.409 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	
39.36 **Glucopy-** **ranose**	 (Text File) Scan 1710 (39.363 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	 (golm) EIQTMS_N12C_SD1_1894.0_1263BK25_Glucopyranose (5TMS)
39.66 **Gulonic acid**	 (Text File) Scan 1726 (39.656 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	 (golm) EIQTMS_N12C_SD1_1964.3_2077BN07_Gulonic acid (6TMS)

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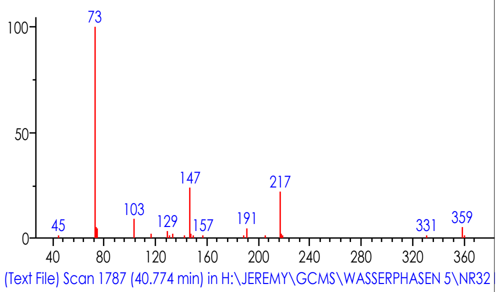
NI 19</td><td></td><td></td></tr>
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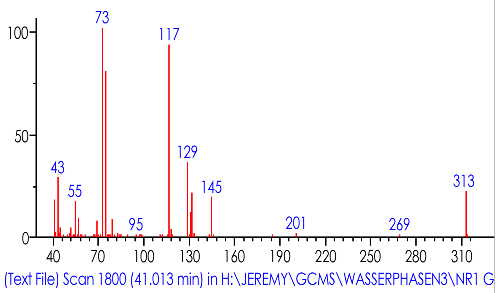
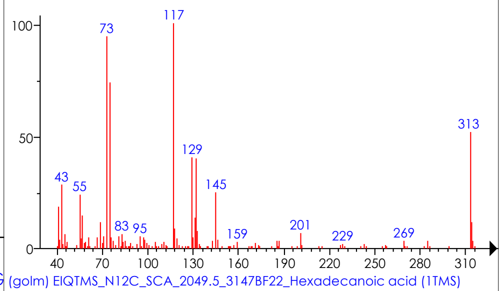
Palmitic acid</td><td></td><td></td></tr>
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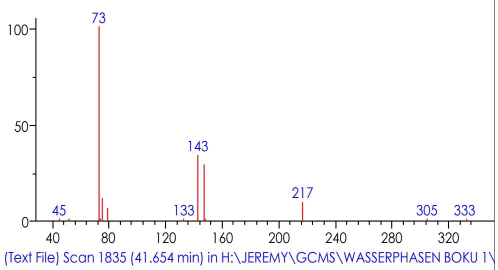
NI 20</td><td></td><td></td></tr>
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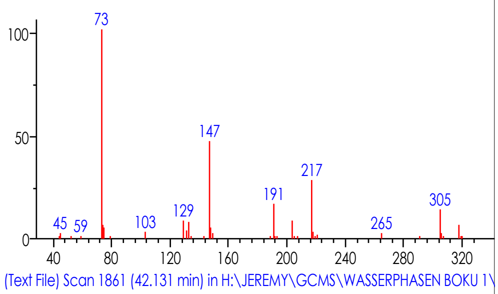
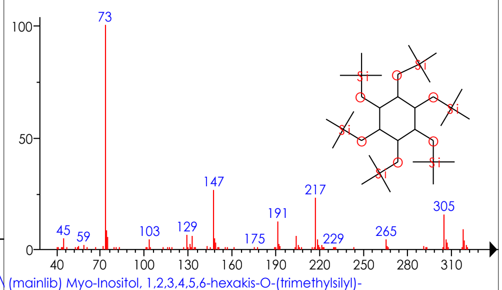
myo-Inositol</td><td></td><td></td></tr>
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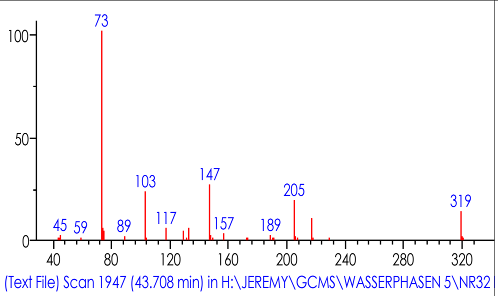
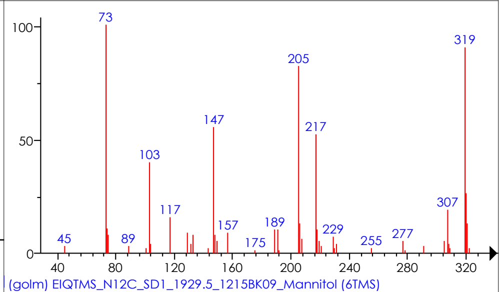
Mannitol</td><td></td><td></td></tr>
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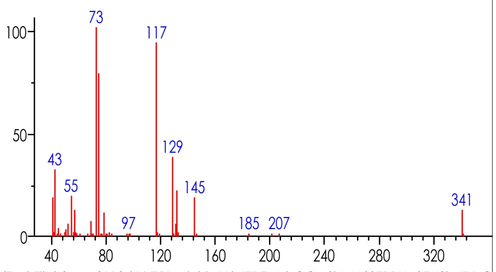
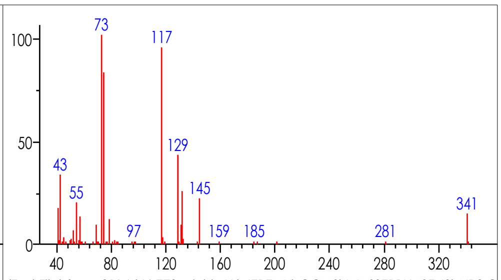
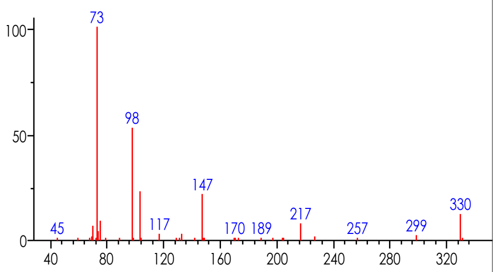
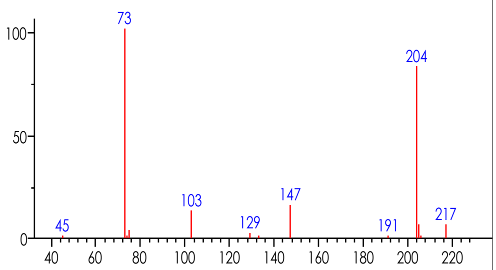
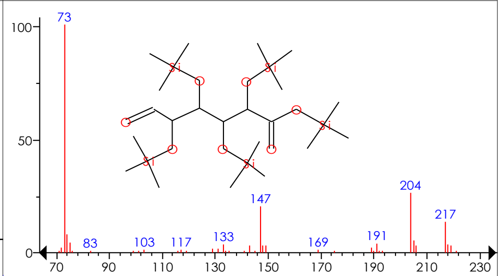
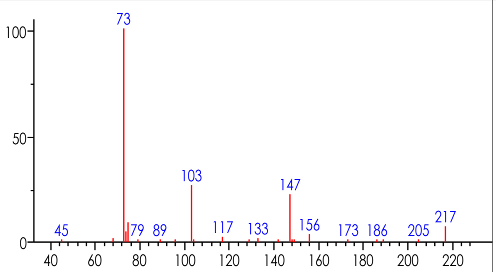
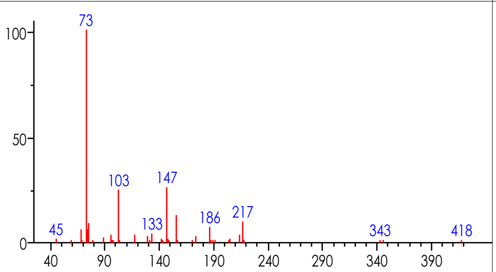
46.75 **Stearic acid**	 (Text File) Scan 2113 (46.751 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR1 G	 (Text File) Scan 2114 (46.770 min) in H:\JEREMY\GCMS\WASSERPHASEN3\NR2 G
47.85 - Not identified 21	 (Text File) Scan 2173 (47.851 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	
49.06 **Galac-turonic acid**	 (Text File) Scan 2233 (48.951 min) in H:\JEREMY\GCMS\WASSERPHASEN 5\NR33	 (mainlib) Galacturonic acid, pentakis(trimethylsilyl)-
52.93 **NI 22**	 (Text File) Scan 2450 (52.930 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	
53.66 **NI 23**	 (Text File) Scan 2490 (53.663 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	

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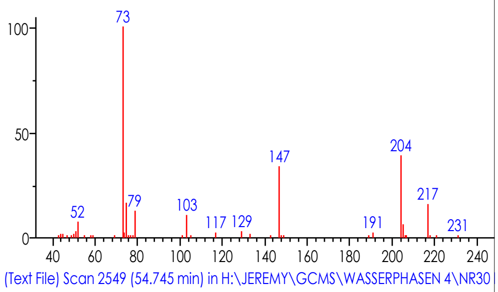
NI 24</td><td>
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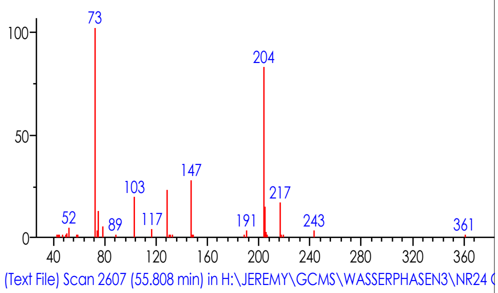
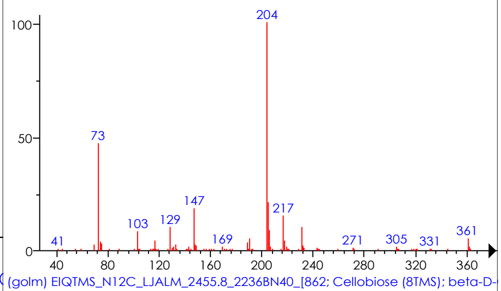
Cellobiose</td><td>
</td><td>
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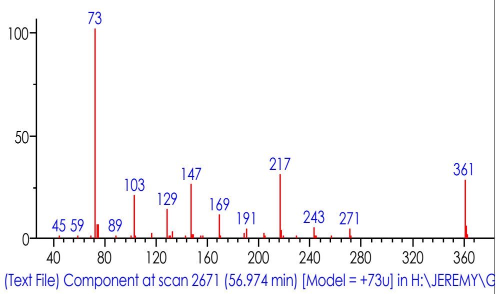
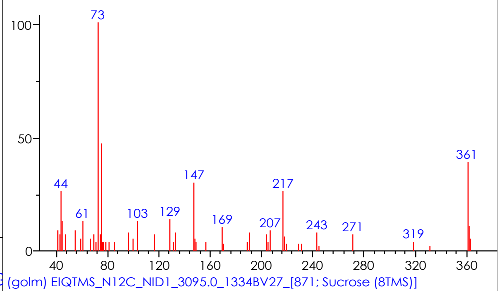
Sucrose</td><td>
</td><td>
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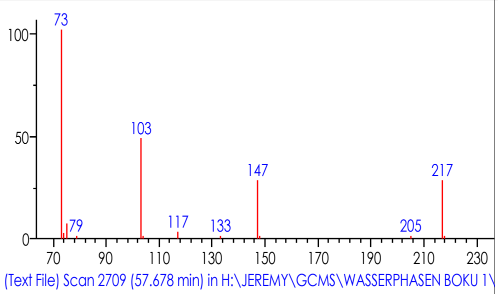
NI 25</td><td>
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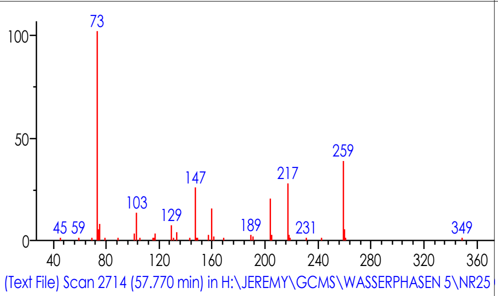
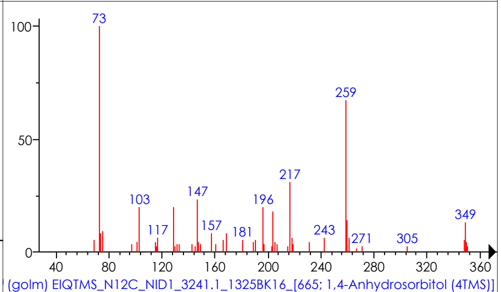
sorbitol</td><td>
</td><td>
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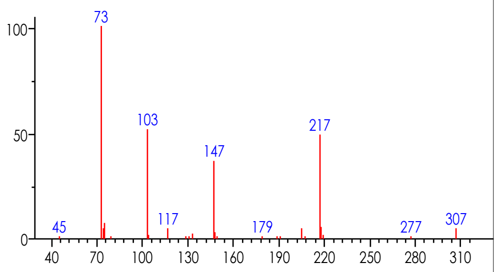
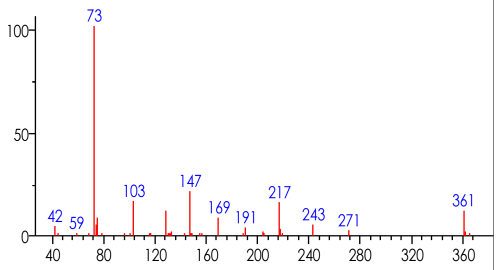
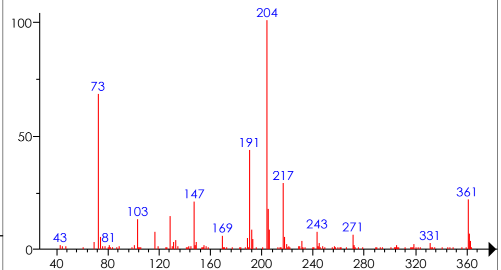
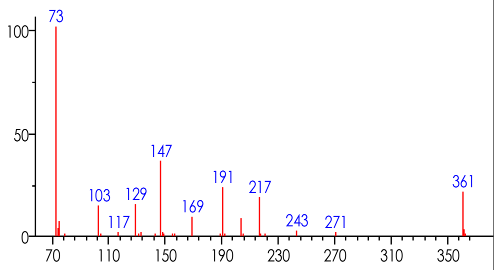
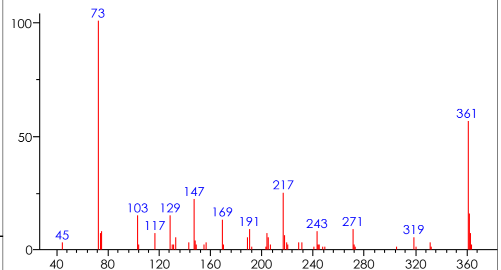
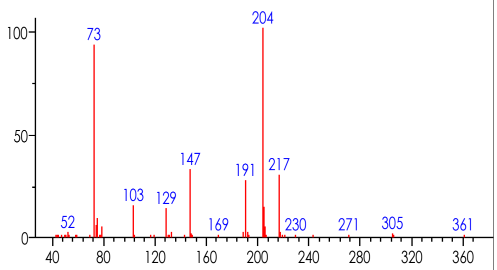
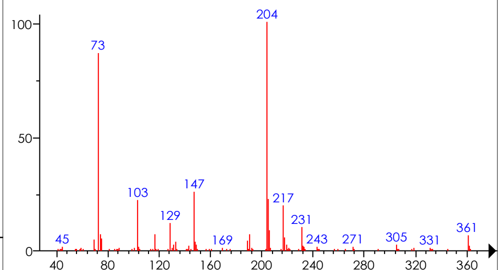
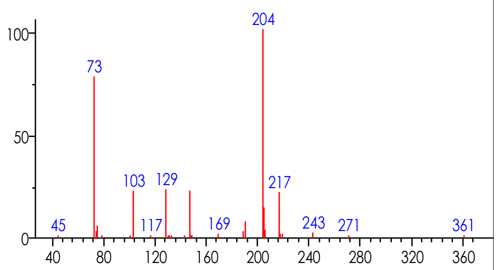
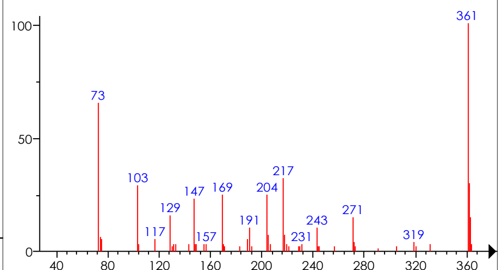
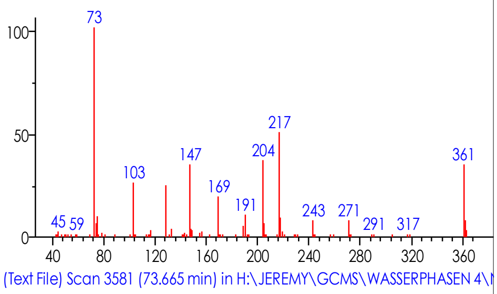
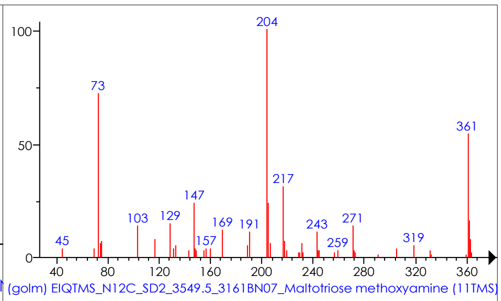
58.06 **NI 26**	 (Text File) Scan 2730 (58.063 min) in H:\JEREMY\GCMS\WASSERPHASEN BOKU 1\	
58.38 **Maltose**	 (Text File) Scan 2721 (57.898 min) in H:\JEREMY\GCMS\WASSERPHASEN 5\	 (golm) EIQTMS_N12C_ATHL_2726.4_3161BN21_[925; Maltose (8TMS)]
59.42 **Trehalose**	 (Text File) Scan 2804 (59.420 min) in H:\JEREMY\GCMS\WASSERPHASEN BO	 (golm) EIQTMS_N12C_NID1_3067.4_1334BV27_[924; Trehalose (8TMS)]
64.64 **Melibiose**	 (Text File) Scan 3089 (64.645 min) in H:\JEREMY\GCMS\WASSERPHASEN 4\NR23	 (golm) EIQTMS_N12C_CMXLP_2451.1_2350BN36_[847; Melibiose (8TMS)]
68.40 **Melizitose**	 (Text File) Scan 3293 (68.385 min) in H:\JEREMY\GCMS\WASSERPHASEN 5\	 (golm) EIQTMS_N12C_SD1_3476.1_2009AU04_Melezitose (11TMS)

73.12 Maltotriose		

6.2. UV spectra experiment 1 and 2

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a)</th><th>UV
b)</th></tr>
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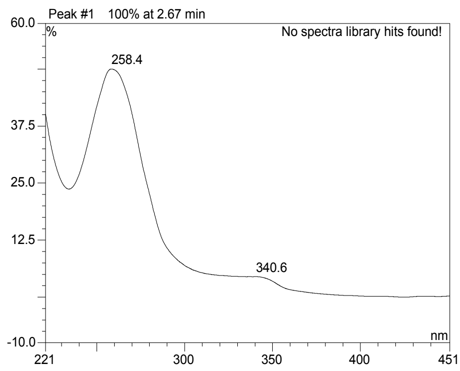
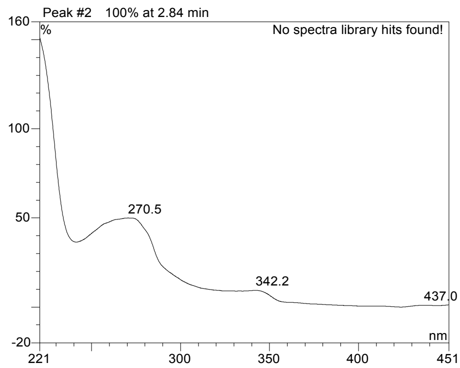
NI 1
2.84 -
NI 2</td><td>
</td><td>
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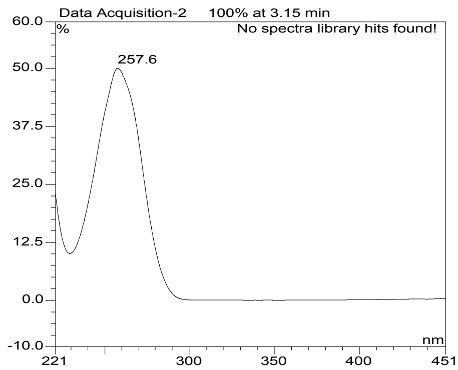
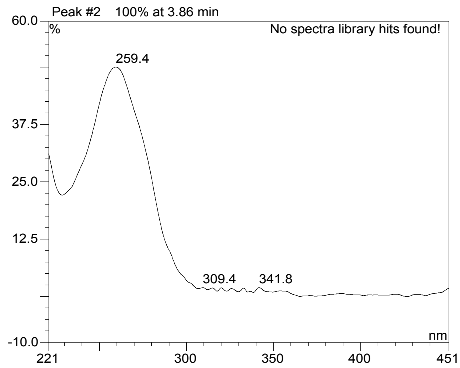
NI 3
3.86 -
NI 4</td><td>
</td><td>
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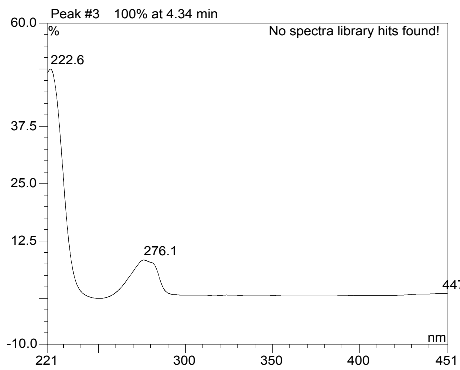
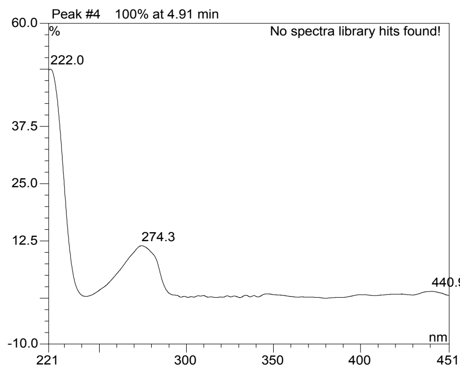
NI 5
4.91 -
Tyrosine</td><td>
</td><td>
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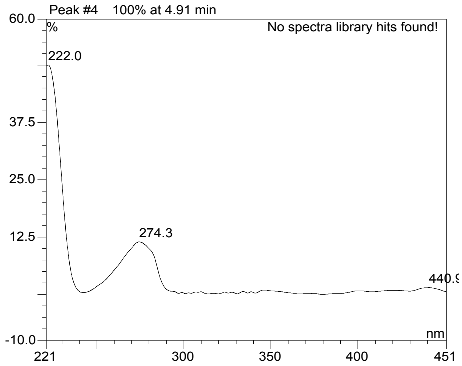
5.82 -
NI 7</td>
<td>
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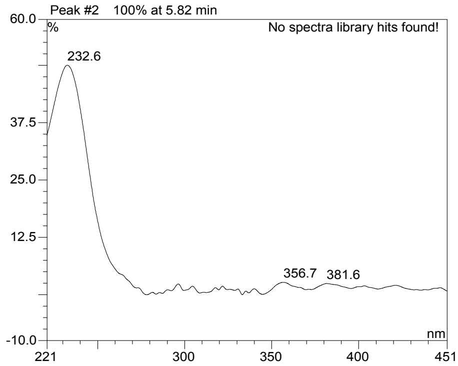
<td>
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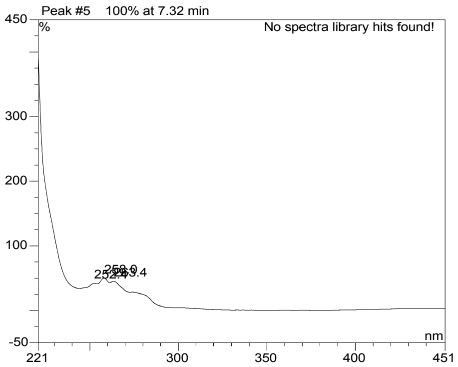
Phenylalanine
7.90 -
NI 8</td>
<td>
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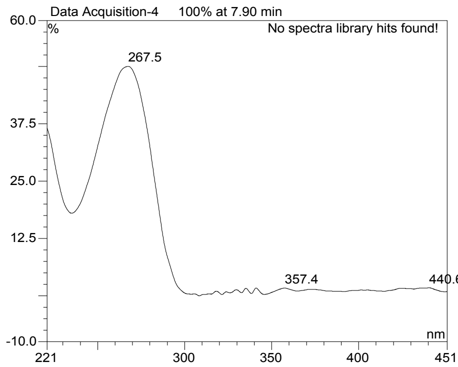
<td>
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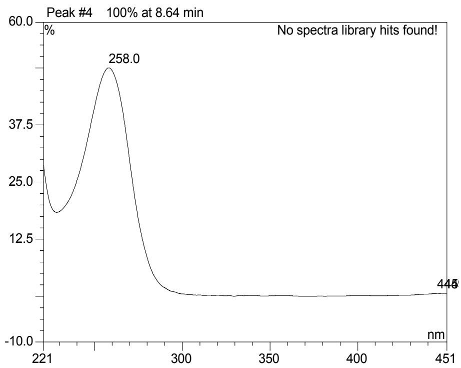
8.79 -
NI 10</td>
<td>
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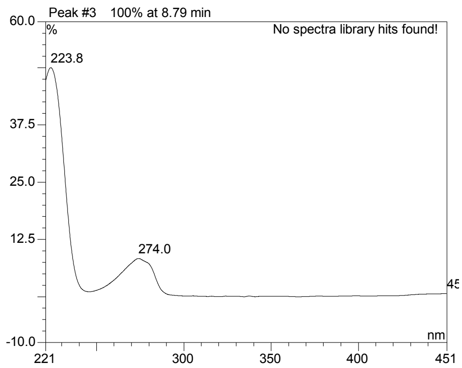
<td>
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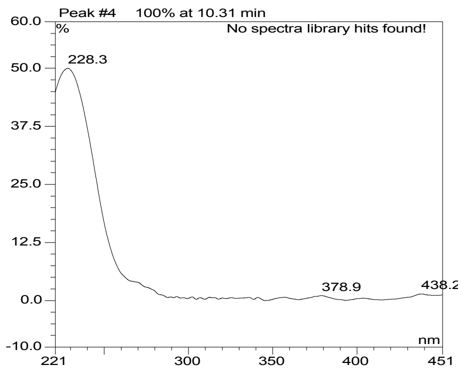
11.59 -
NI 12</td>
<td>
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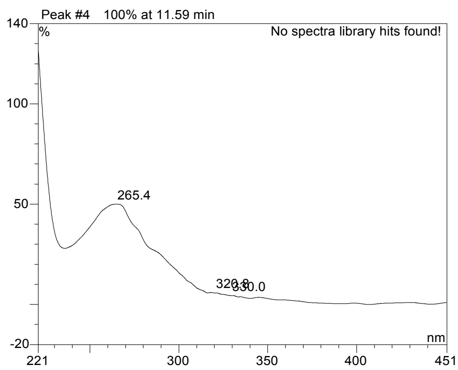
<td>
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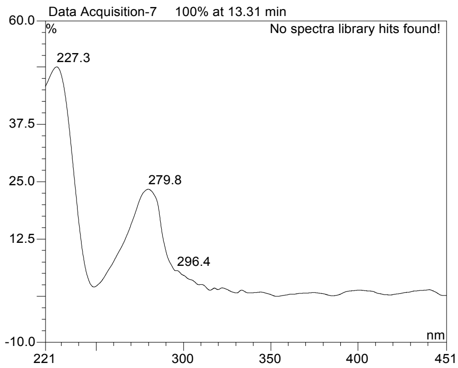
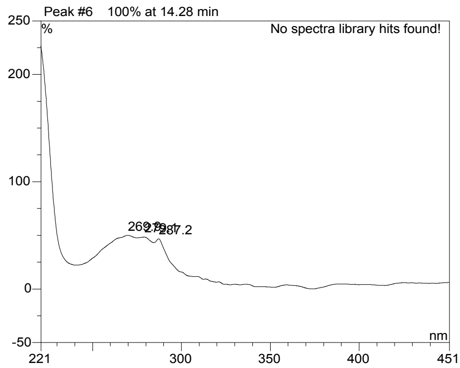
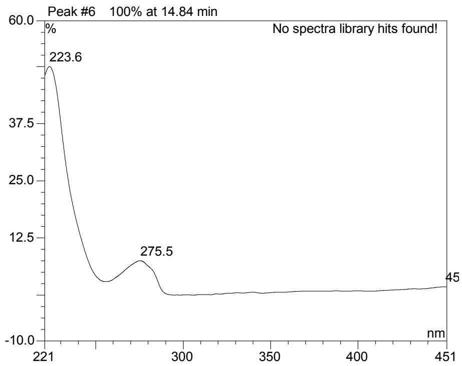
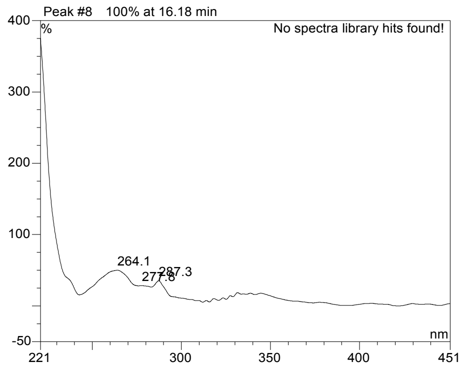
13.31 -
NI 13
14.28 -
Tryptophane



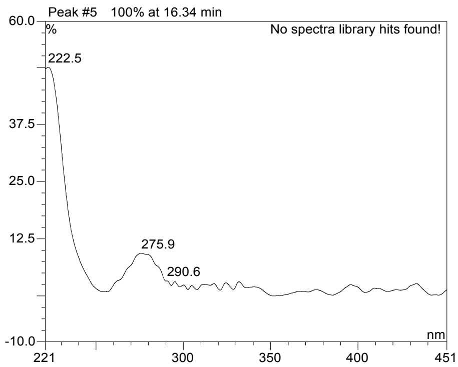


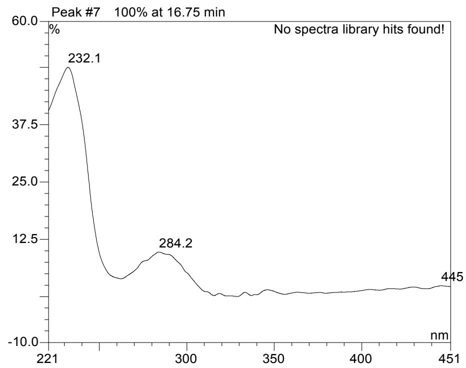
14.84 -
NI 14
16.19 -
NI 15



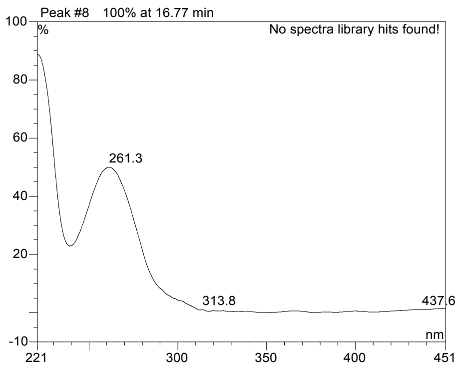


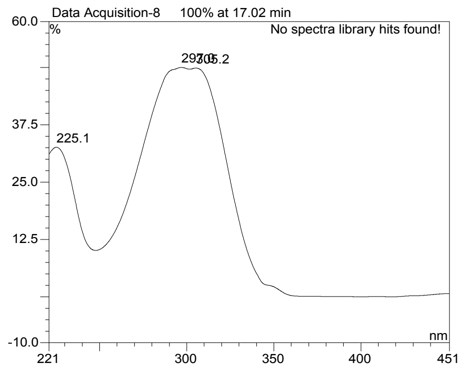
16.35 -
NI 16
16.68 -
NI 17





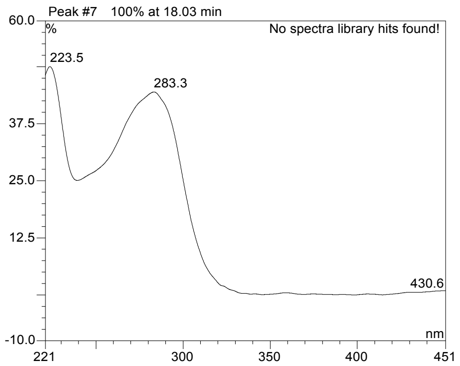
16.77 -
NI 18
17.02 -
NI 19

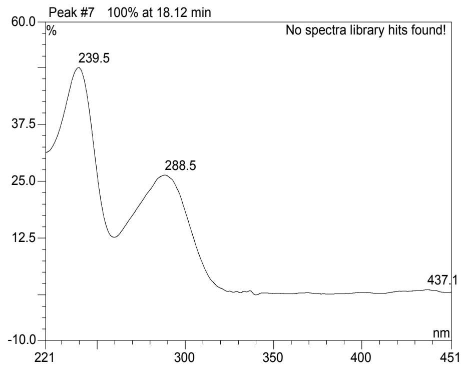




18.03 - **NI 20**

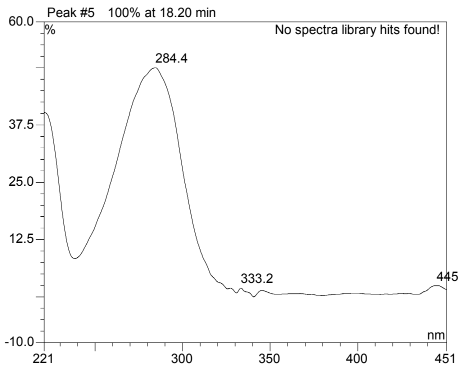
18.12 - **NI 21**

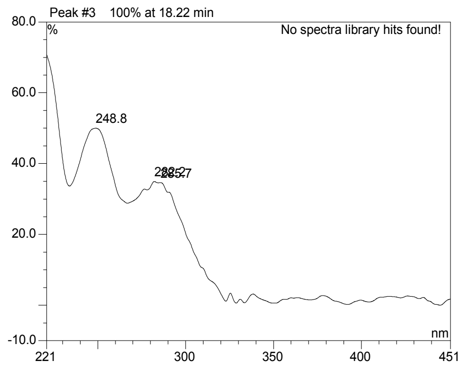




18.20 - **NI 22**

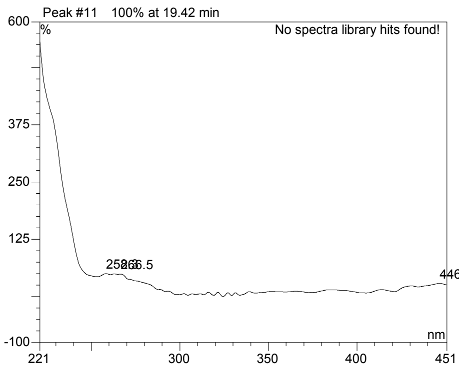
18.22 - **Vanillic acid**

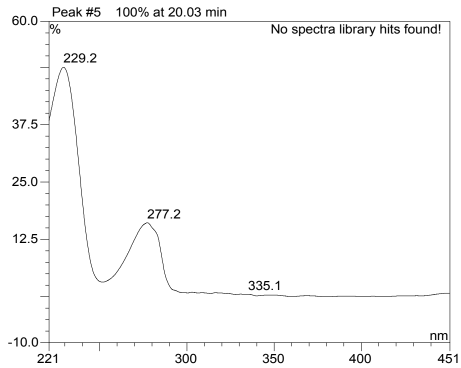




19.42 - **NI 24**

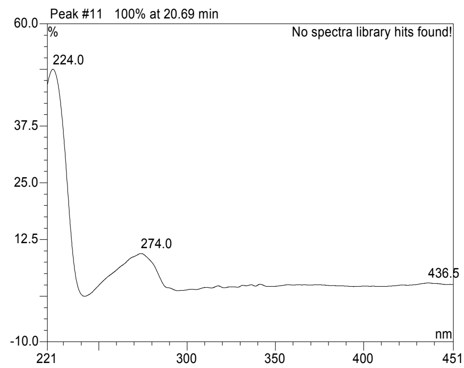
20.03 - **Phenylacetic acid**

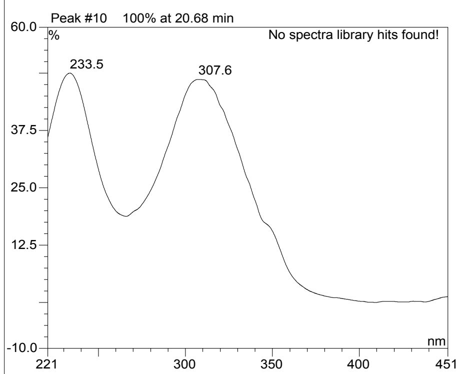




20.69 - **Syringic acid**

20.63 - **NI 25**





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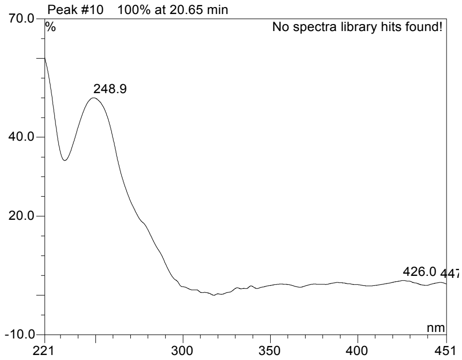
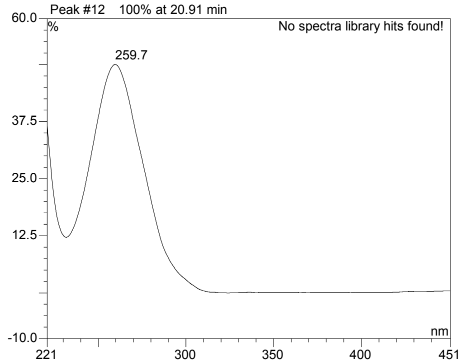
NI 27</td>
<td></td>
<td></td>
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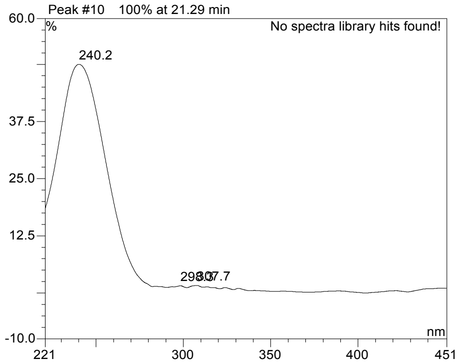
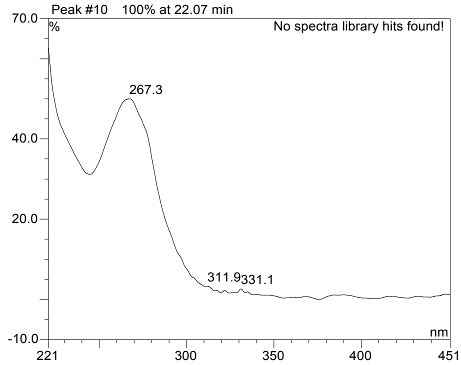
22.17 -
NI 29</td>
<td></td>
<td></td>
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22.63 -
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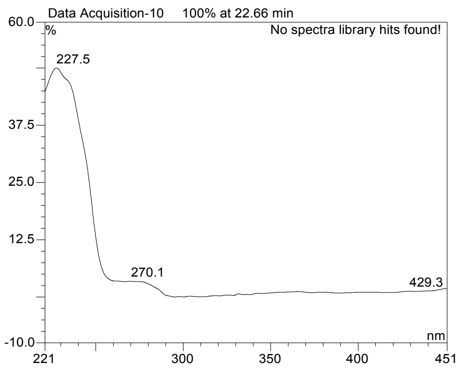
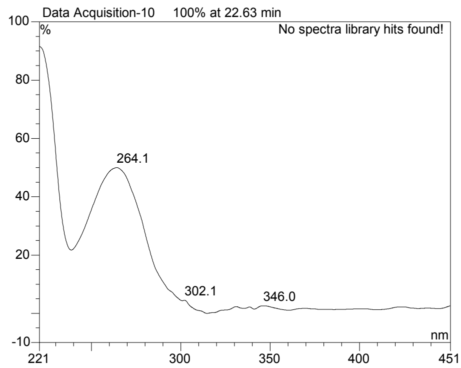
<td></td>
<td></td>
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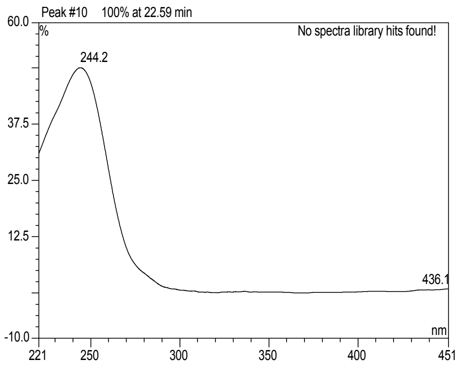
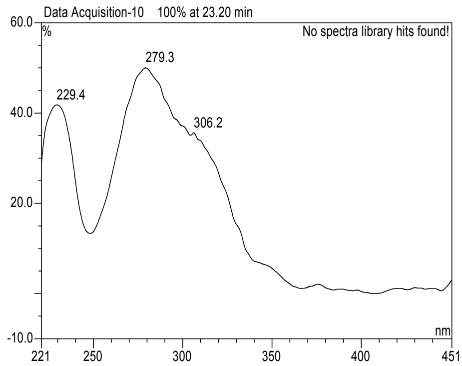
<td></td>
<td></td>
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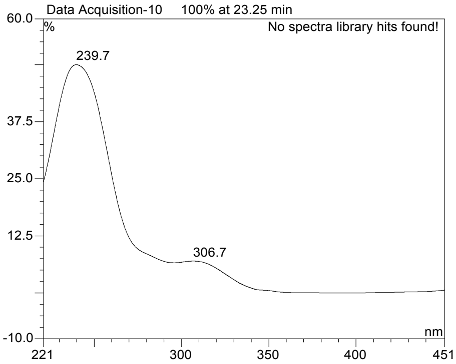
<td>
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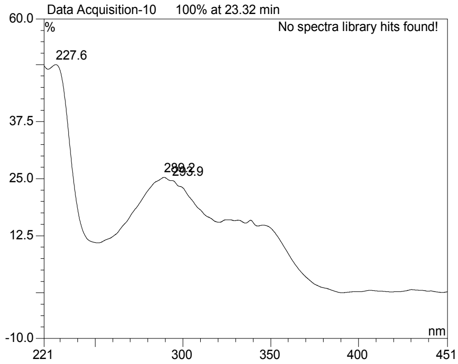
<td>
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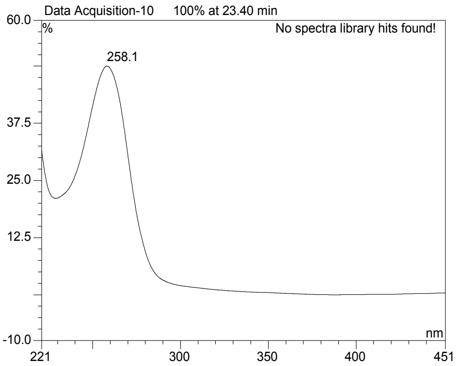
<td>
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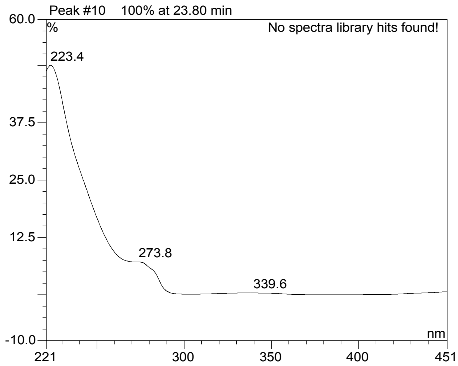
<td>
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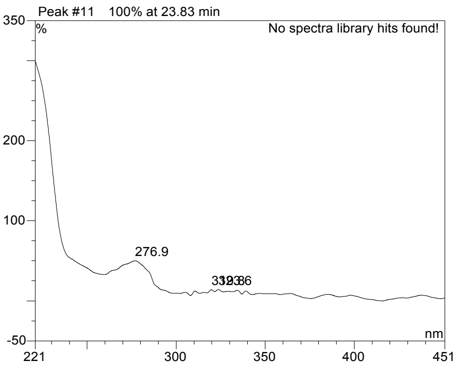
<td>
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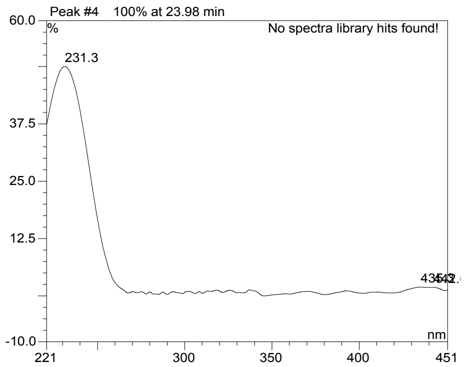
<td>
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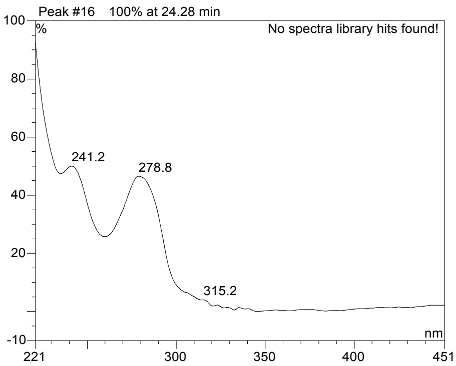
<td>
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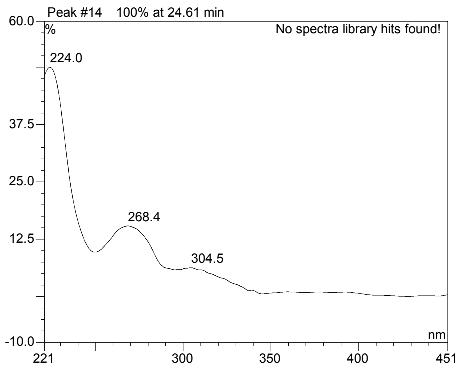
<td>
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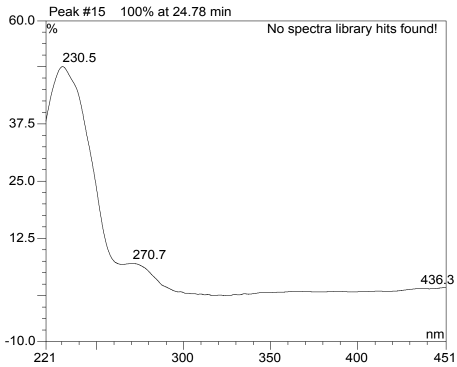
<td>
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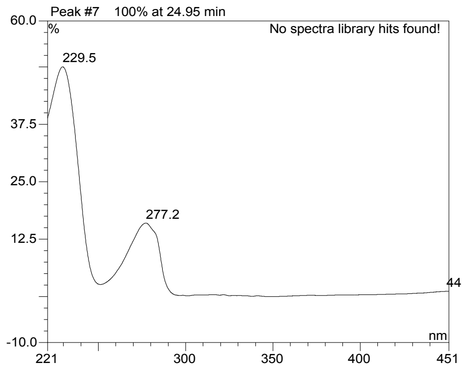
<td>
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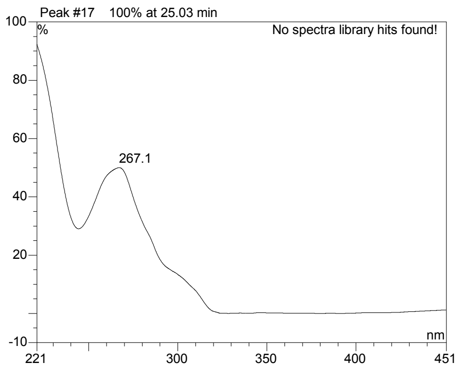
<td>
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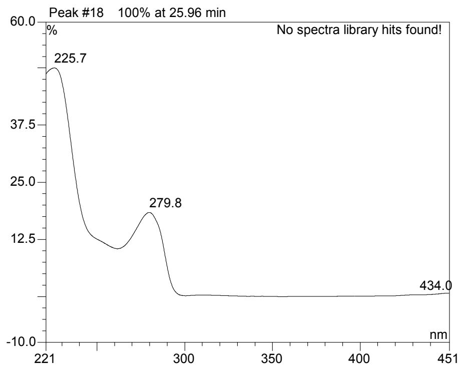
<td>
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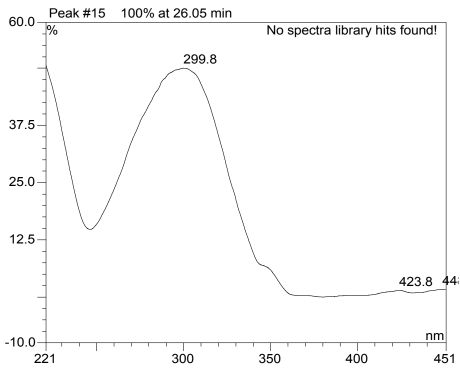
<td>
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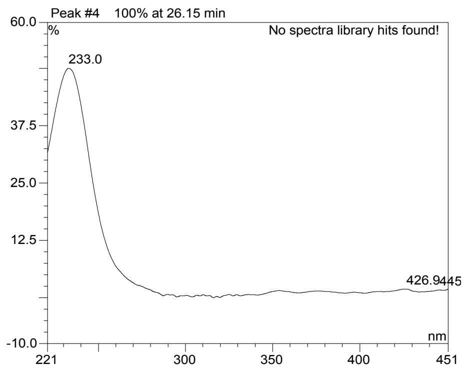
<td>
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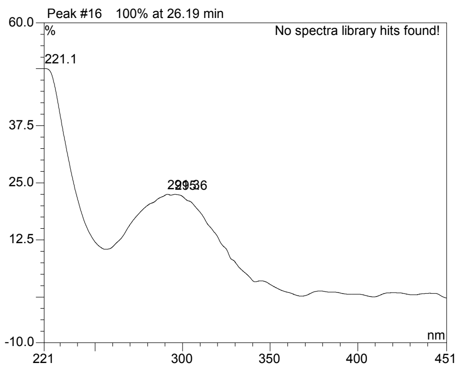
NI 50</td>
<td>
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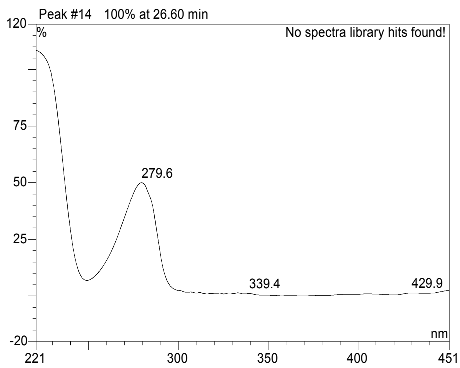
<td>
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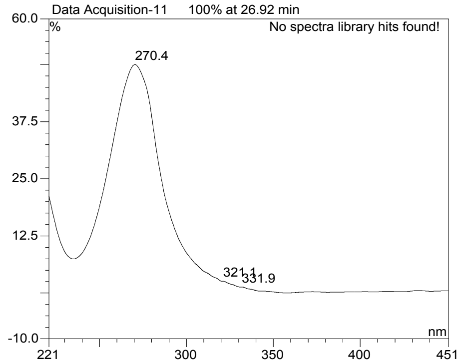
<td>
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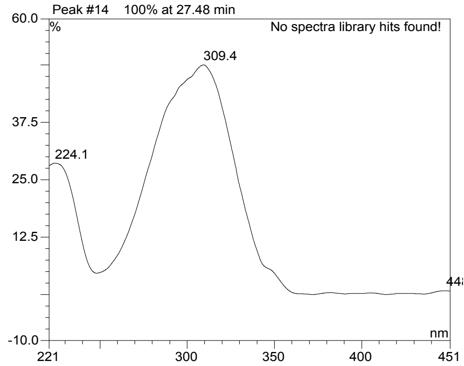
<td>
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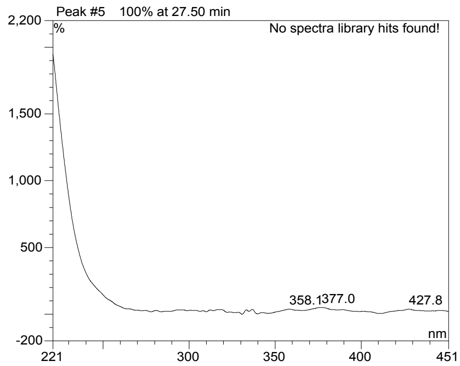
NI 53</td>
<td>
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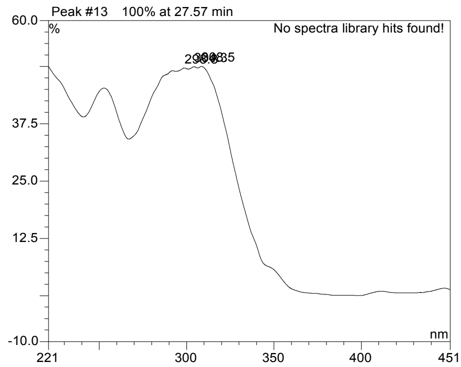
<td>
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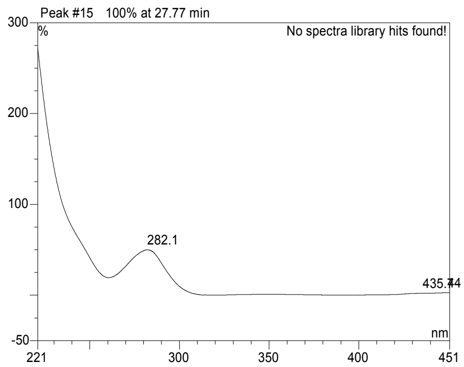
NI 55</td>
<td>
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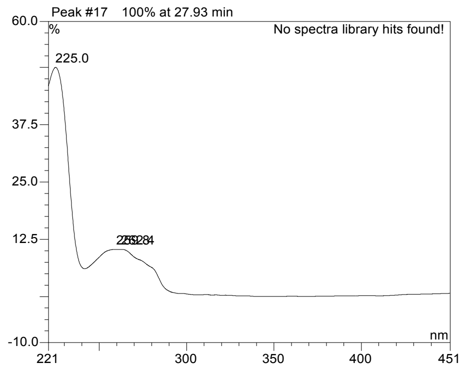
<td>
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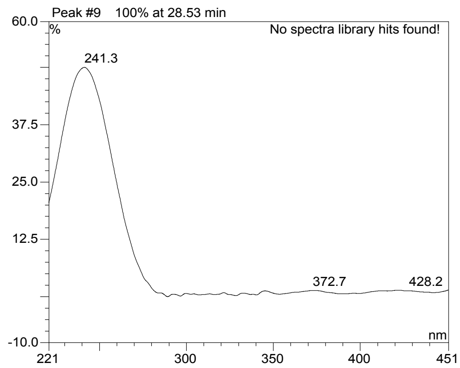
NI 57</td>
<td>
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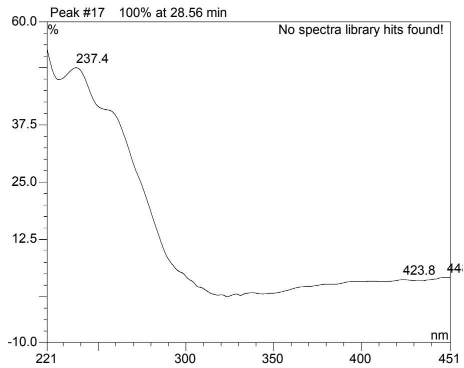
<td>
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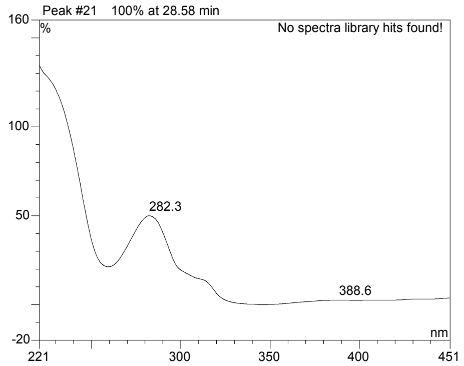
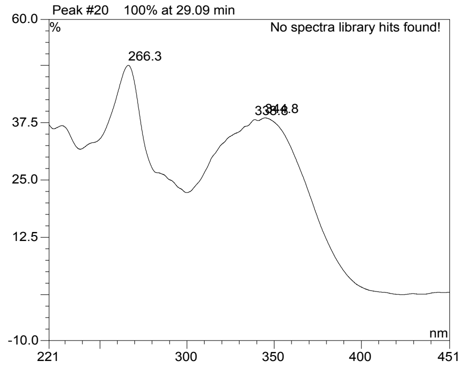
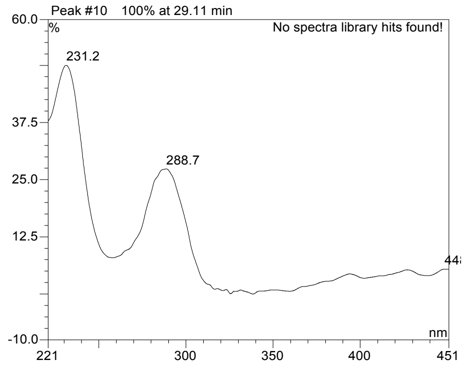
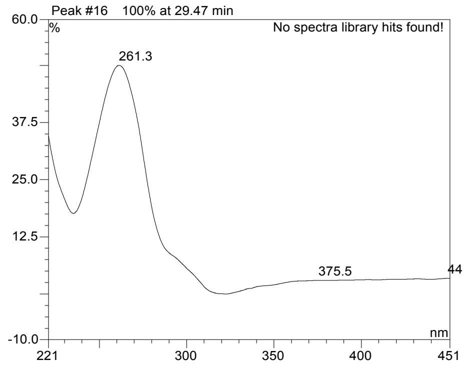
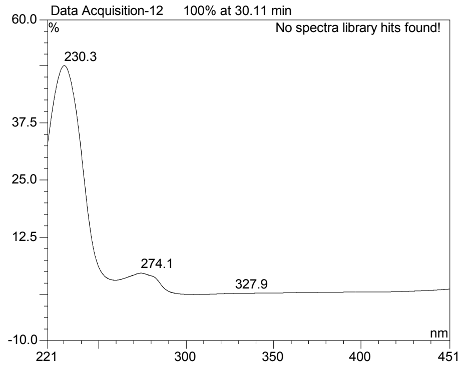
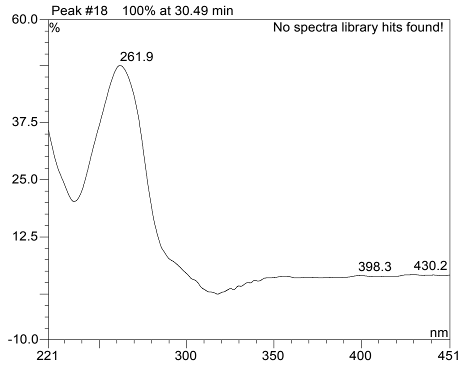
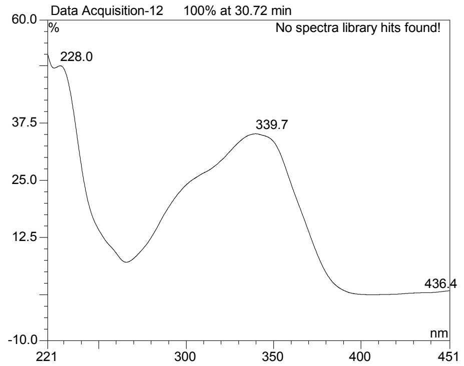
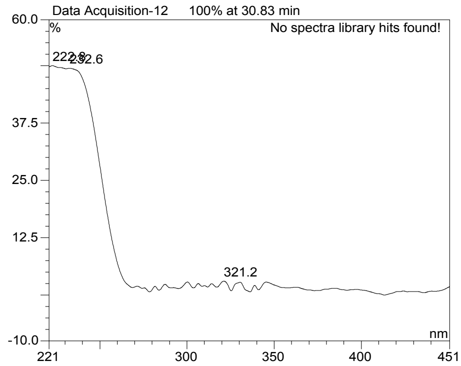
28.58 - NI 58 29.09 - **Kaempferol glycoside**		
29.11 - **NI 59** 29.47 - **NI 60**		
30.11 - **Benzoic acid** 30.49 - **NI 61**		
30.72 - **Ferulic acid** 30.83 - **NI 62**		

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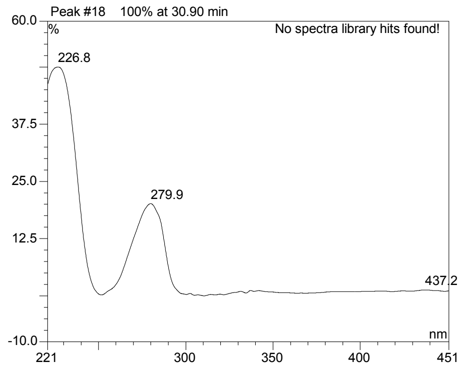
31.06 -
NI 64</td>
<td>
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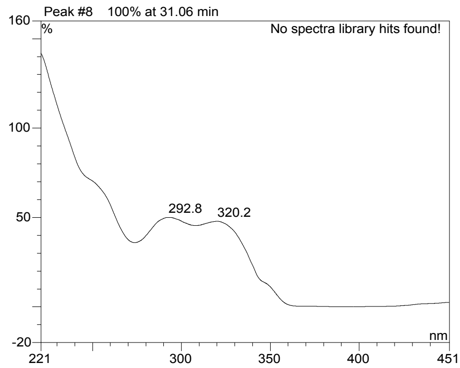
<td>
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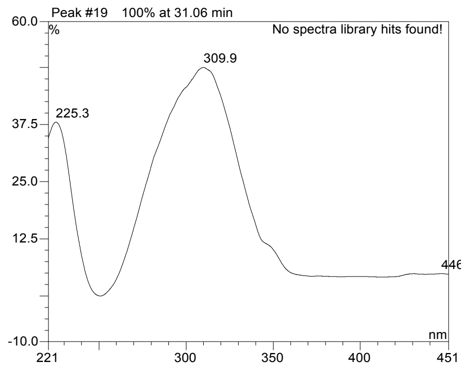
<td>
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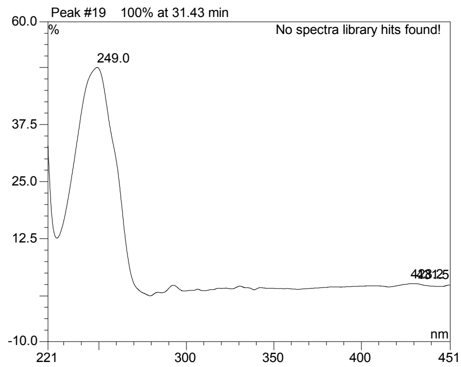
<td>
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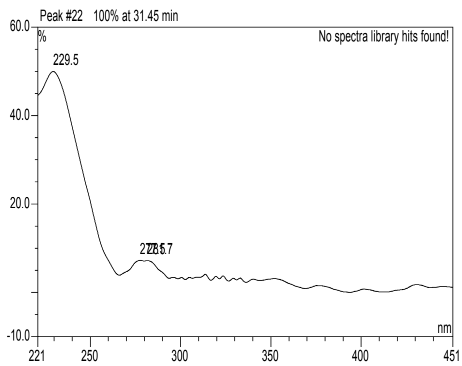
31.82 -
NI 67</td>
<td>
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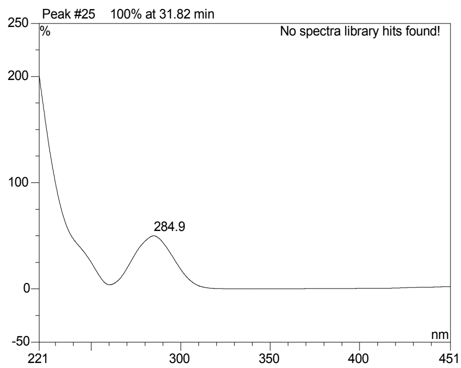
<td>
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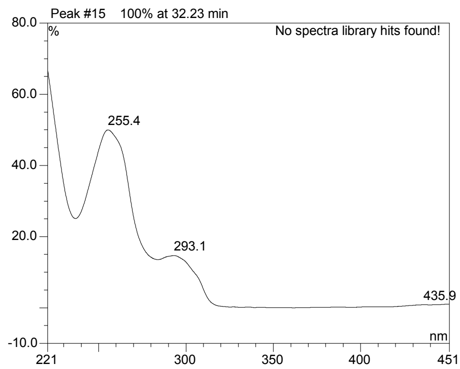
<td>
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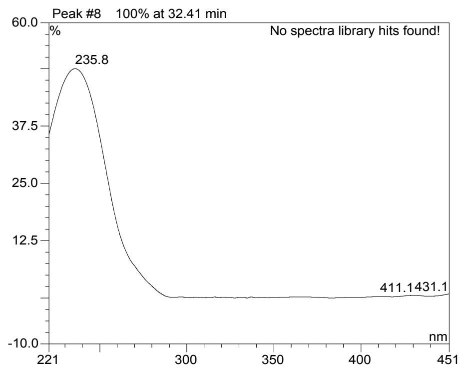
<td>
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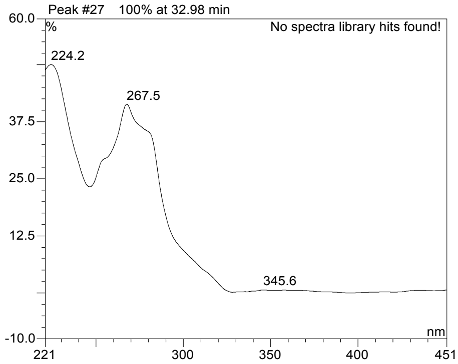
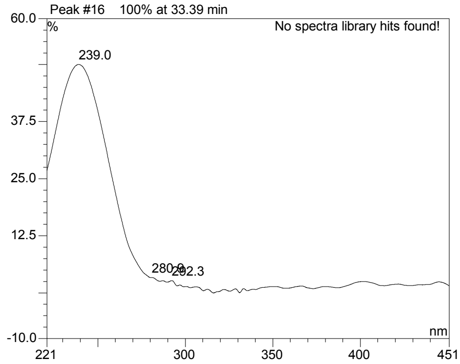
32.98 -

NI 70

33.39 -

NI 71



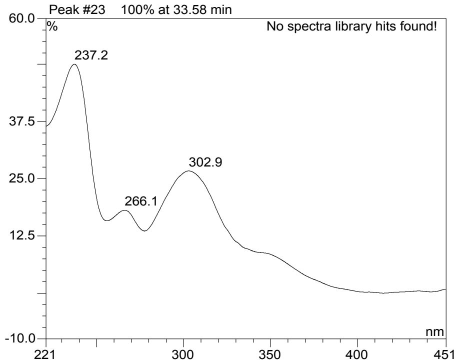


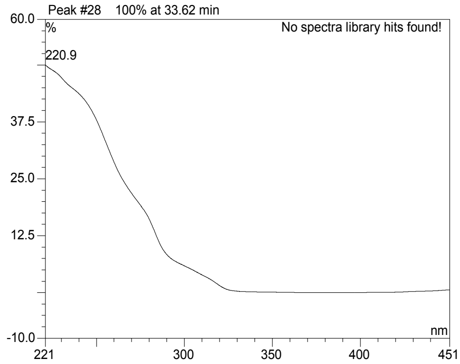
33.58 -

NI 72

33.62 -

NI 73



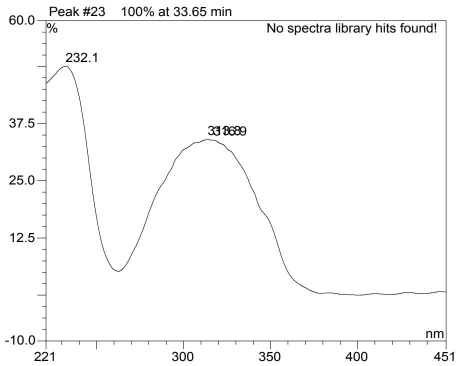


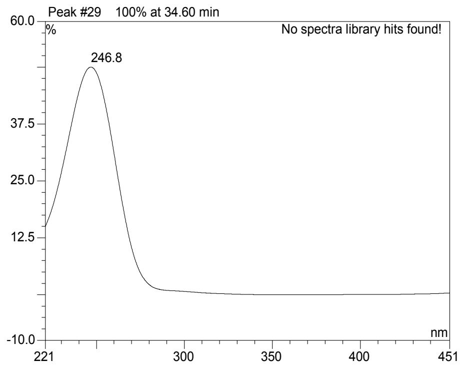
33.65 -

NI 73a

34.60 -

NI 74



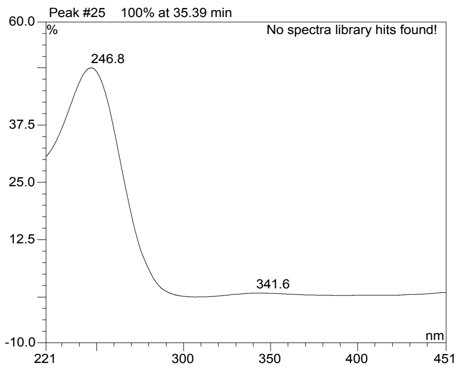


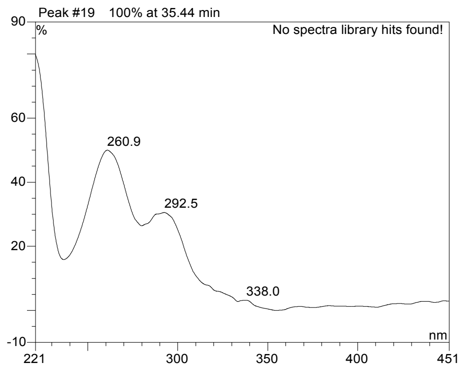
35.39 -

NI 75

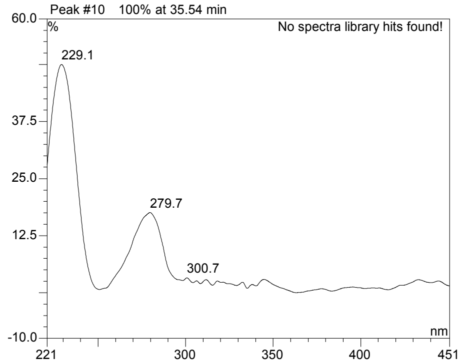
35.44 -

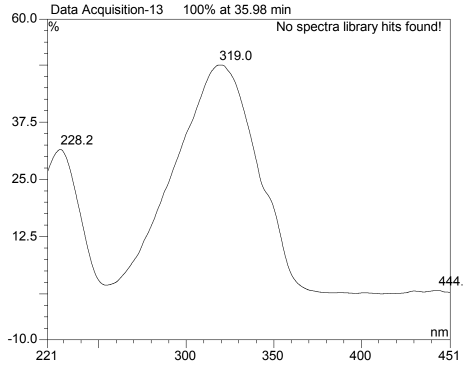
NI 76



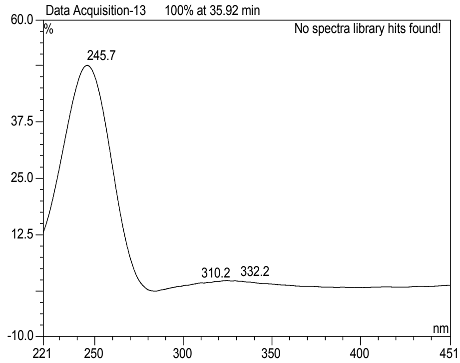


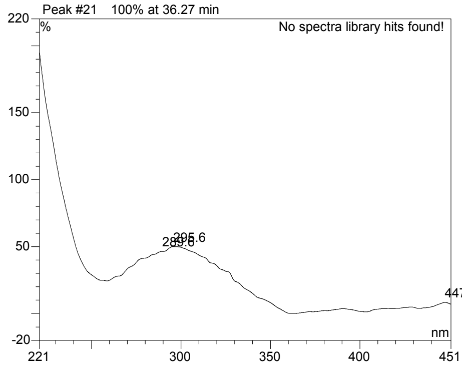
35.54 -
NI 77
35.70 -
Sinapic acid



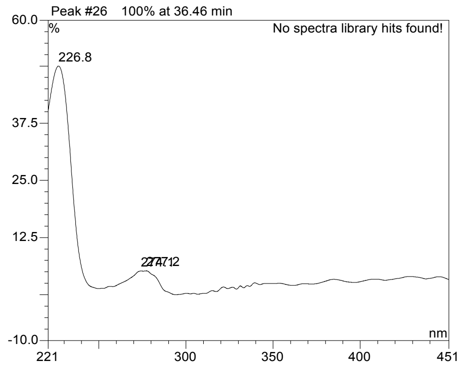


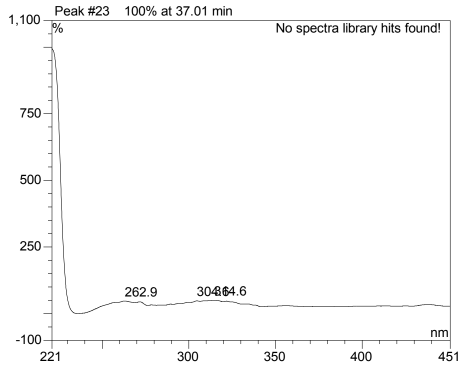
35.83 -
NI 78
36.27 -
NI 79



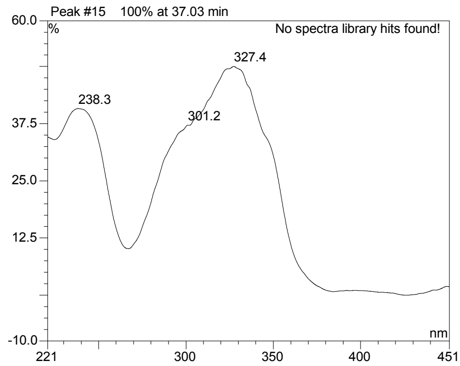


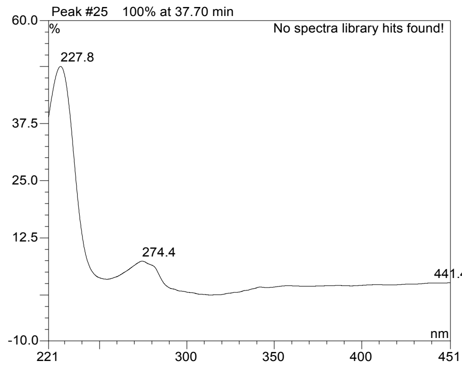
36.47 -
NI 80
37.01 -
NI 81



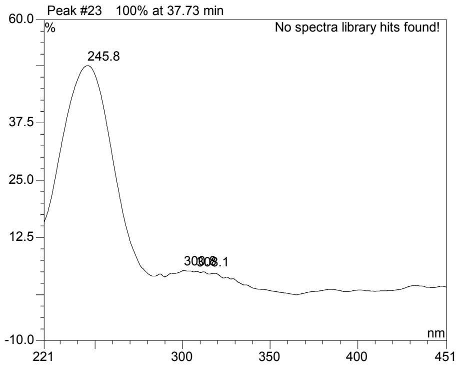


37.03 -
Chlorogenic acid
37.70 -
NI 82

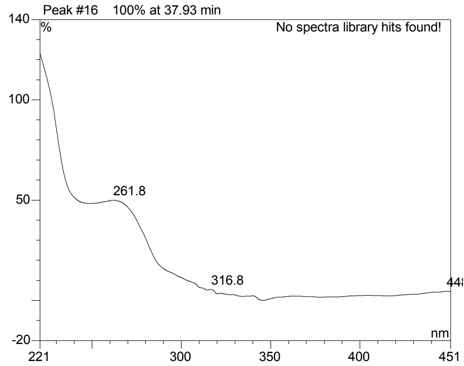




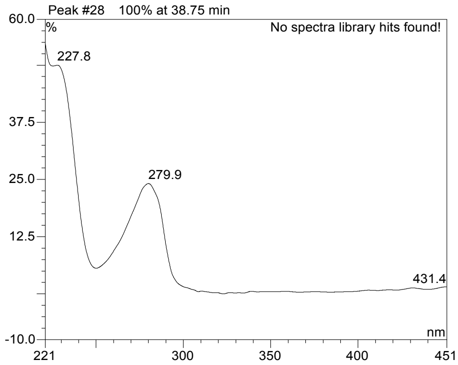
37.73 - **NI 83**



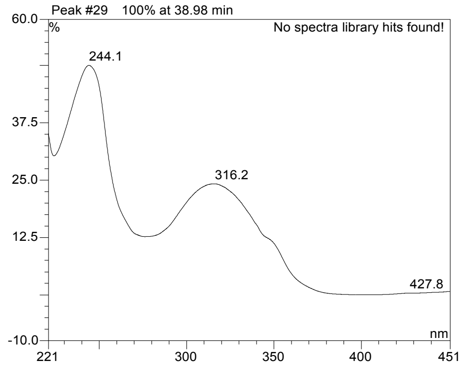
37.93 - **NI 84**



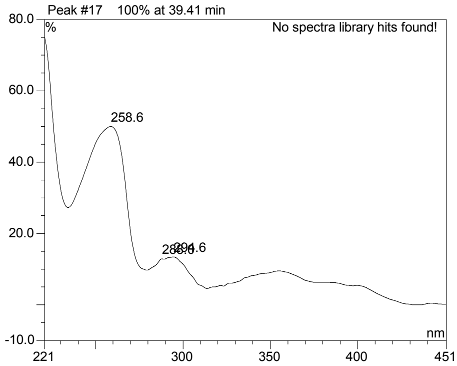
38.79 - **NI 85**



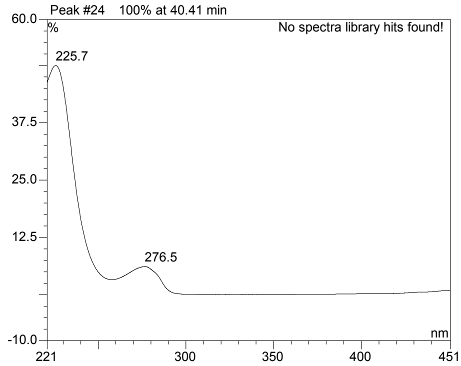
39.10 - **NI 86**



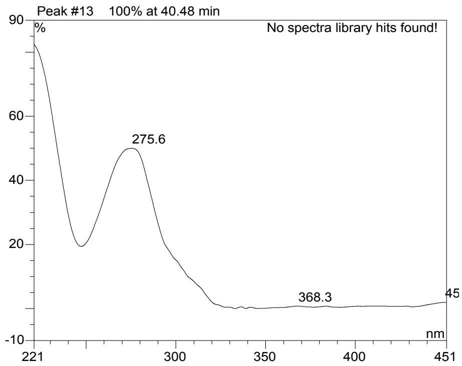
39.41 - **NI 87**



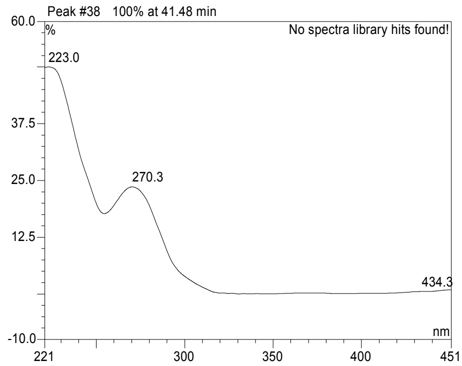
40.41 - **NI 88**



40.48 - **NI 89**



41.48 - **NI 90**



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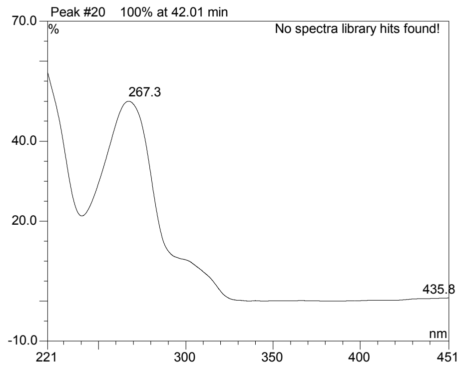
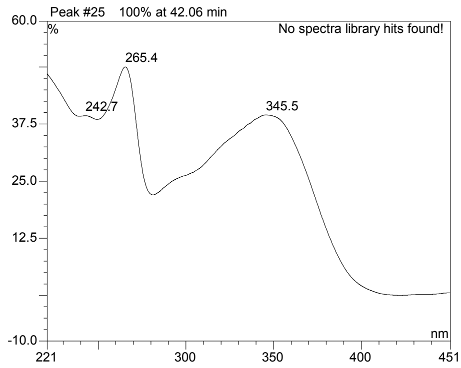
Kaempferol glycoside</td><td>
</td><td>
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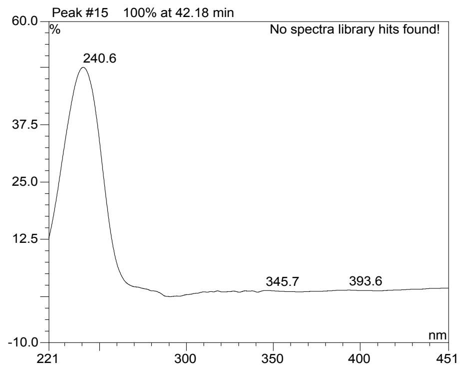
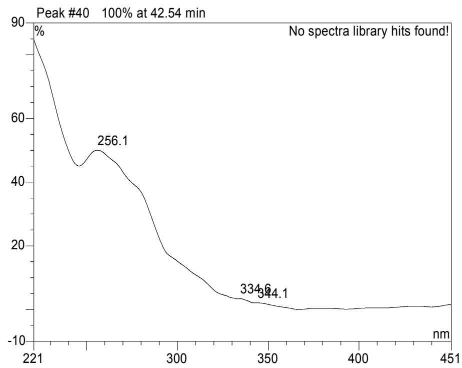
NI 93</td><td>
</td><td>
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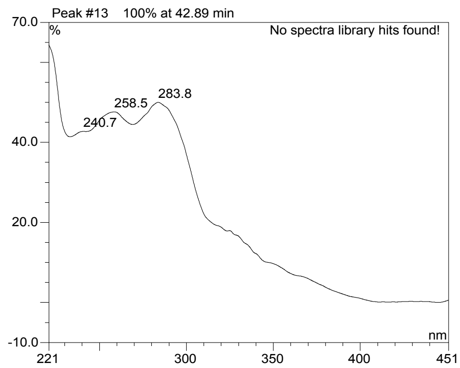
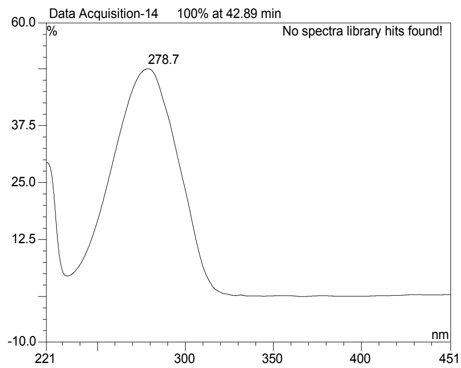
Cinnamic acid</td><td>
</td><td>
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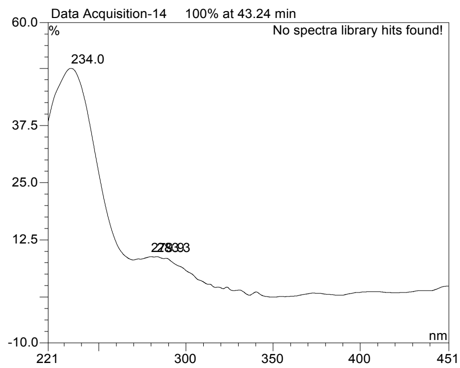
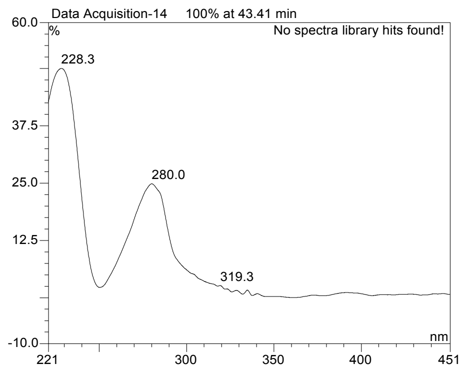
NI 96</td><td>
</td><td>
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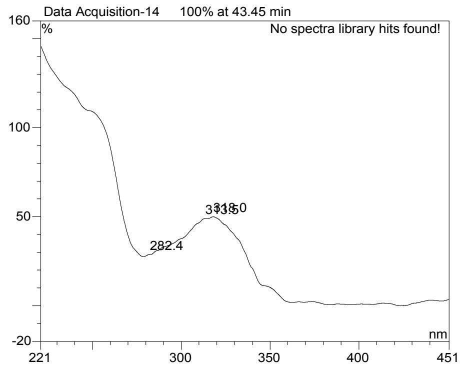
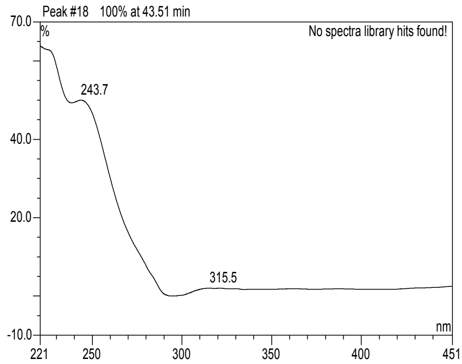
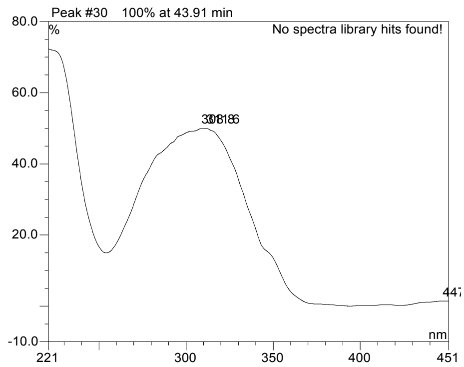
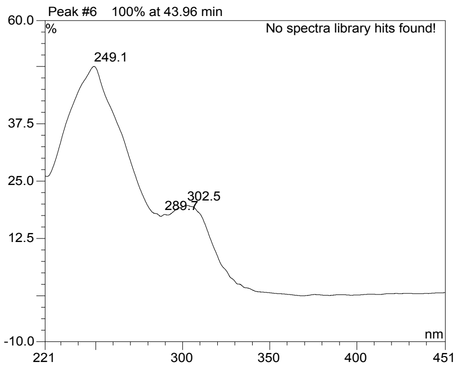
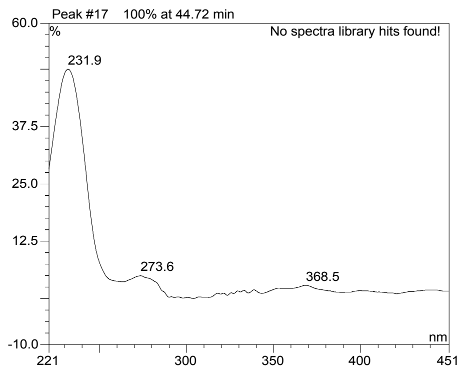
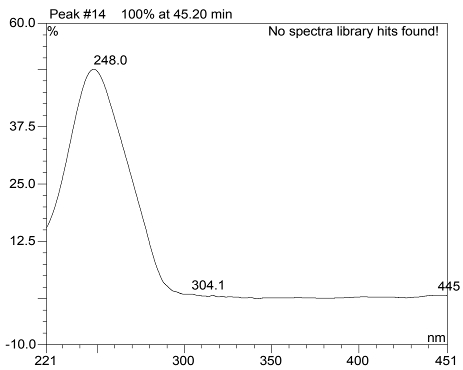
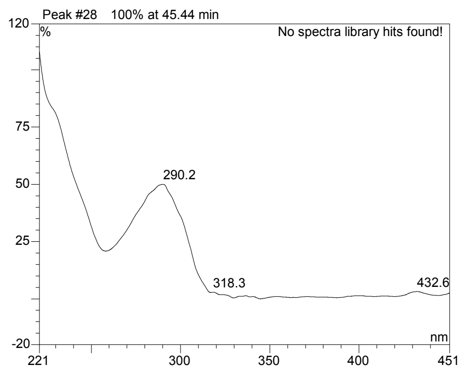
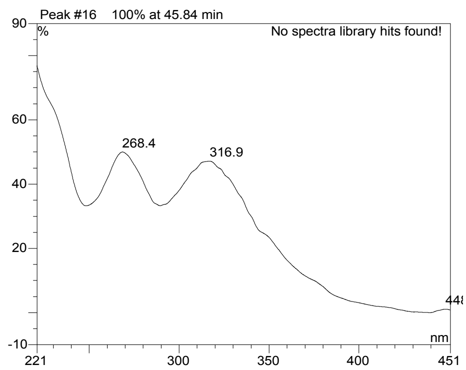
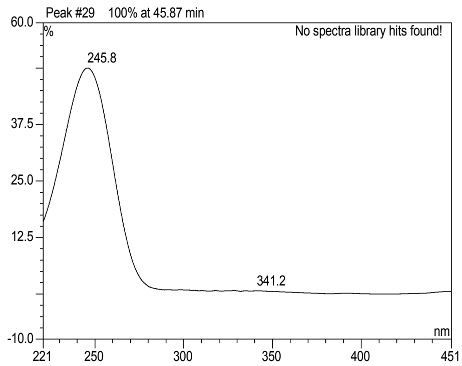
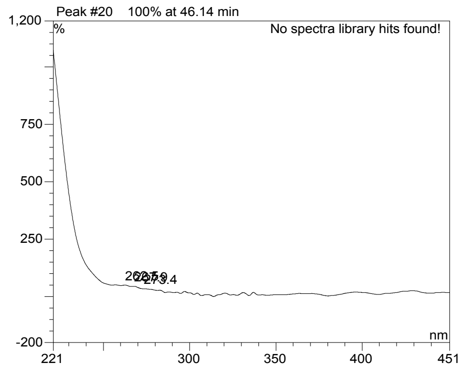
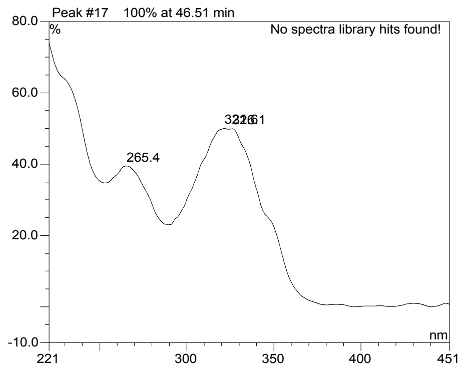
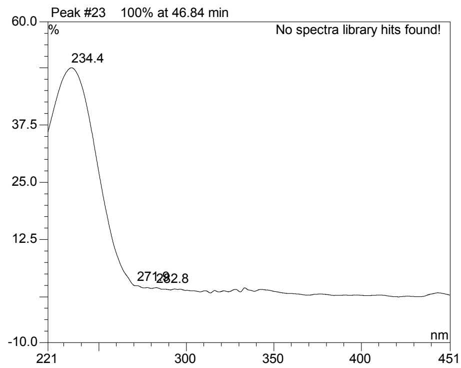
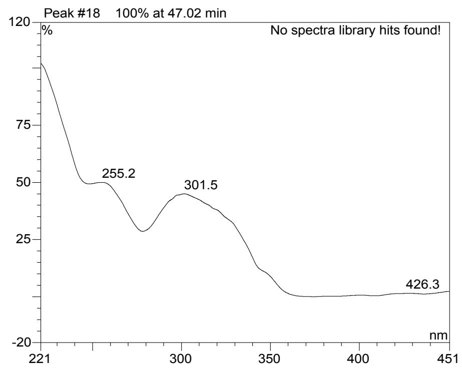
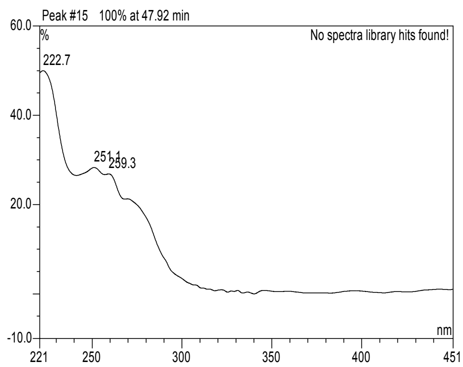
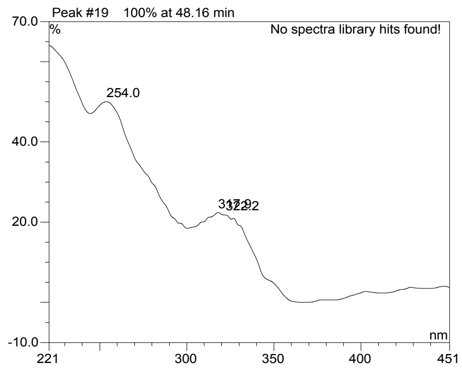
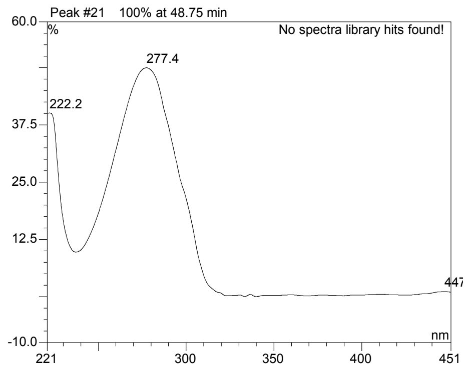
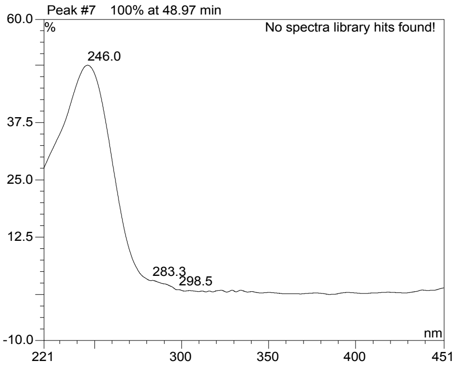
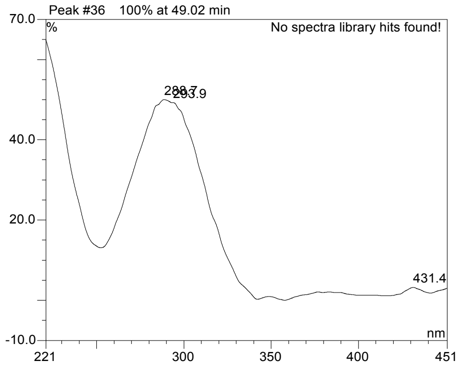
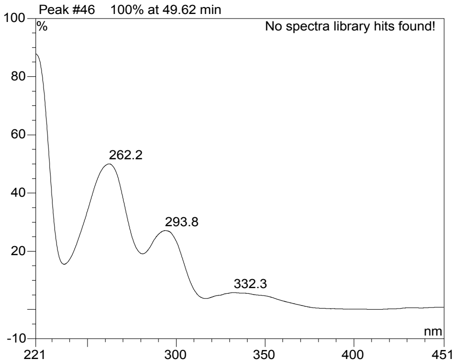
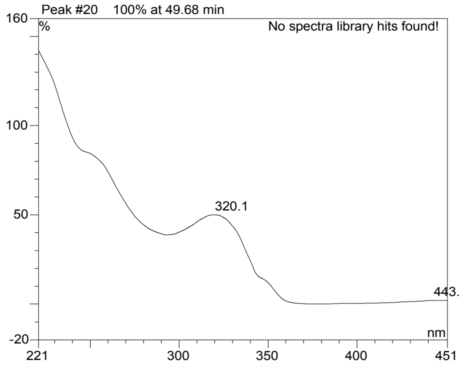
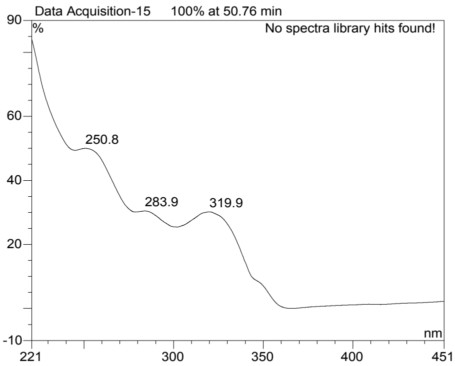
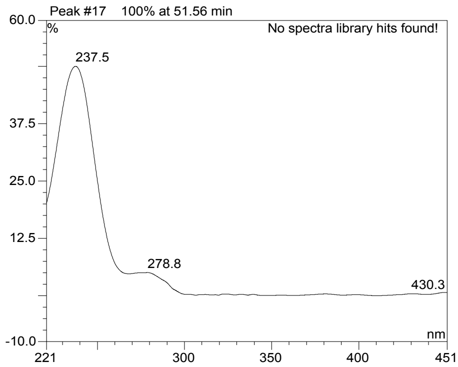
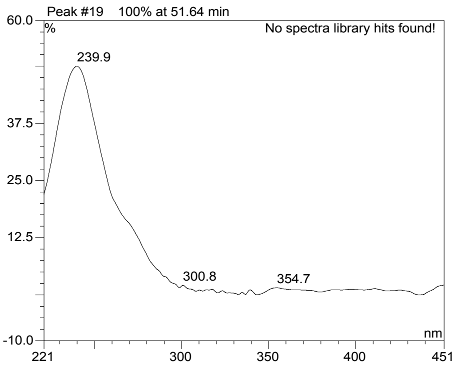
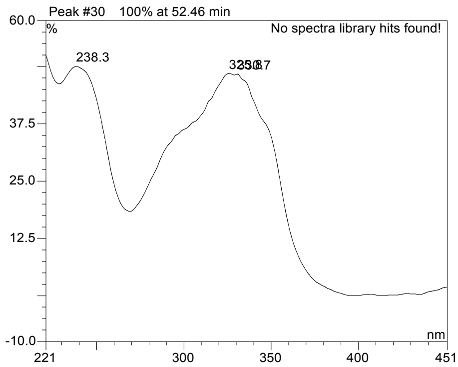
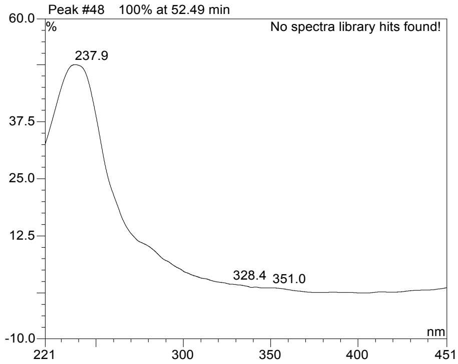
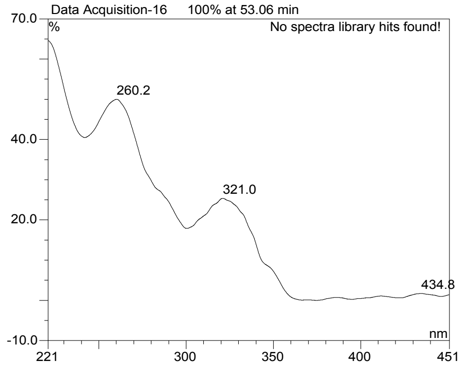
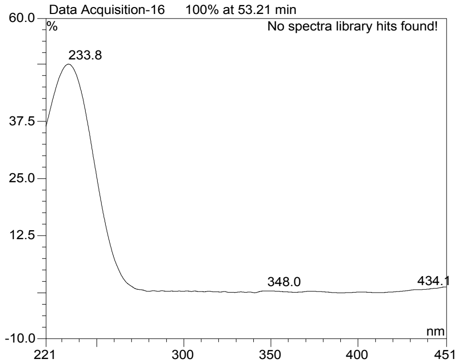
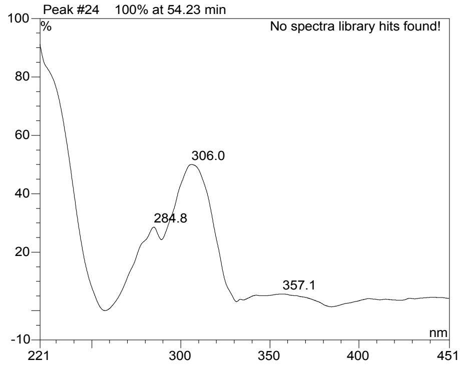
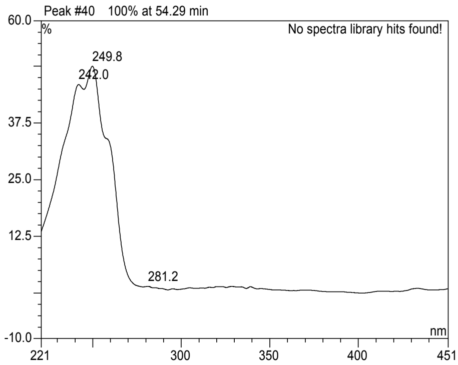
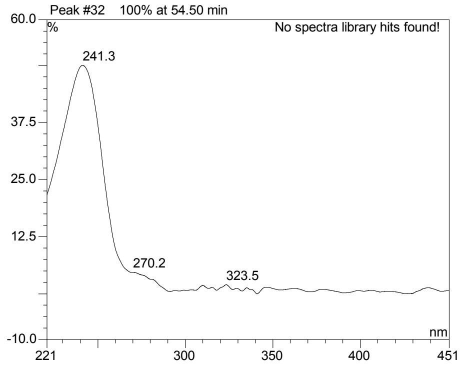
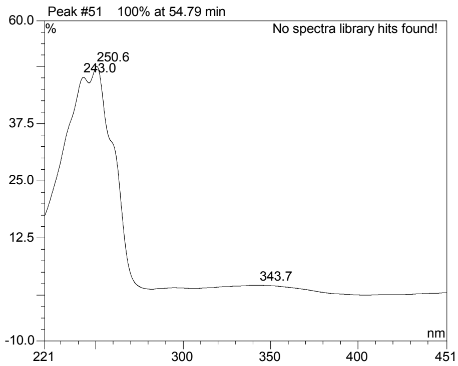
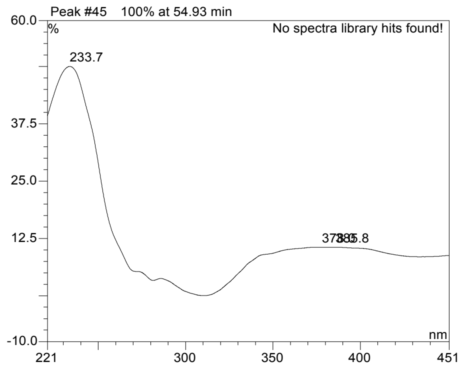
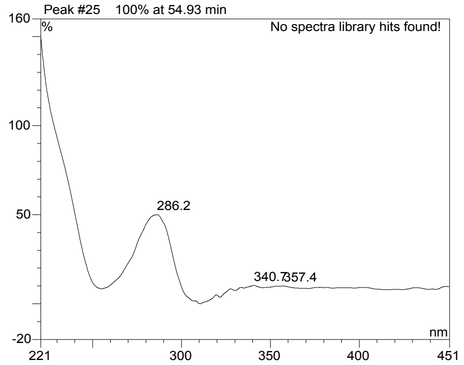
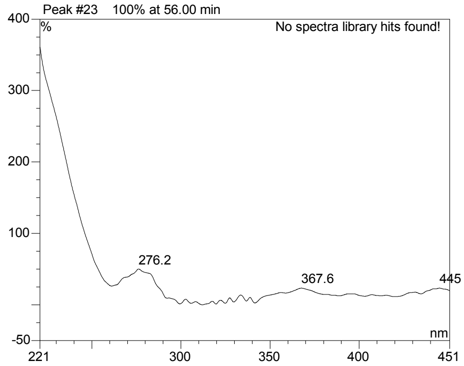
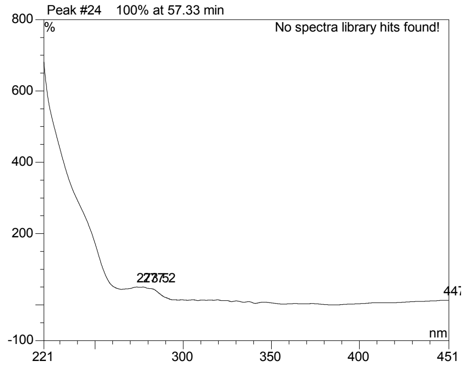
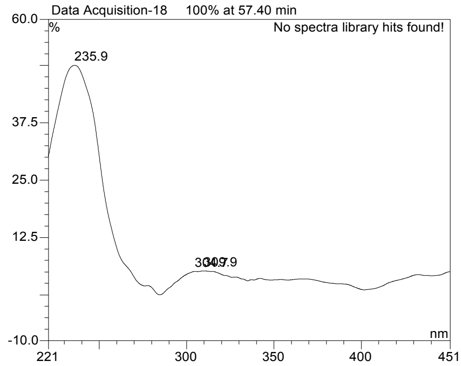
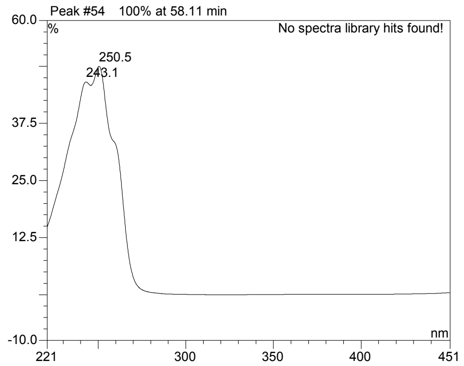
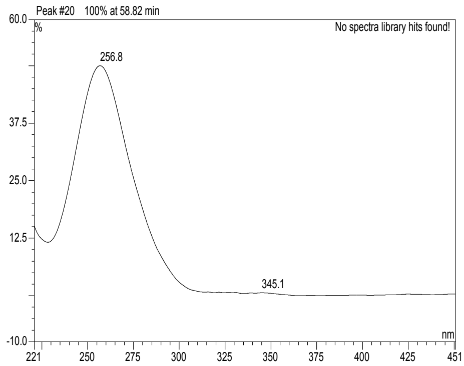
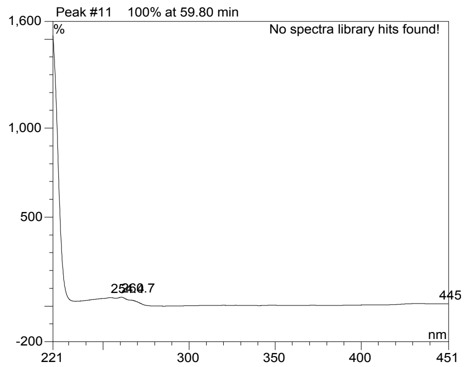
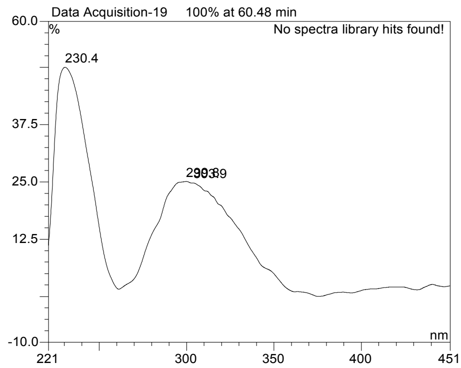
43.45 - **NI 97** 43.53 - **NI 98**		
43.91 - **NI 99** 43.96 - **NI 100**		
44.72 - **NI 101** 45.20 - **NI 102**		
45.44 - **NI 103** 45.84 - **NI 104**		

45.87 - **NI 105** 46.14 - **NI 106**		
46.51 - **NI 107** 46.84 - **NI 108**		
47.02 - **NI 109** 47.87 - **NI 110**		
48.16 - **NI 111** 48.75 - **NI 112**		

48.97 - **NI 113** 49.06 - **NI 114**		
49.62 - **NI 115** 49.68 - **NI 116**		
50.76 - **NI 117** 51.56 - **NI 118**		
51.64 - **NI 119** 52.46 - **Cinnamic acid derivative**		

52.59 - **NI 120** 53.06 - **NI 121**		
53.21 - **NI 122** 54.23 - **Pisatin**		
54.29 - **NI 123** 54.50 - **NI 124**		
54.79 - **NI 125** 54.92 - **NI 126**		

54.93 - **NI 127** 56.00 - **NI 128**		
57.33 - **NI 129** 57.39 - **NI 130**		
58.11 - **NI 131** 58.82 - **NI 132**		
59.80 - **NI 133** 60.51 - **NI 134**		

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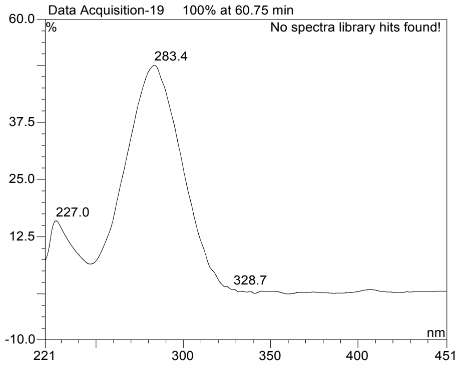
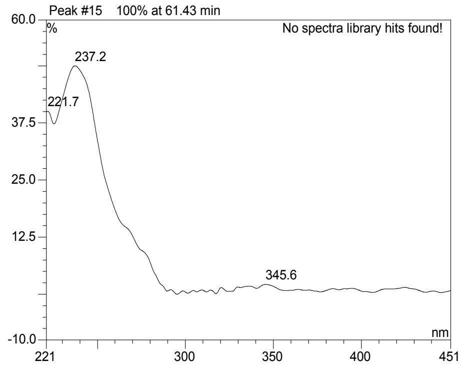
NI 135

61.43 -

NI 136



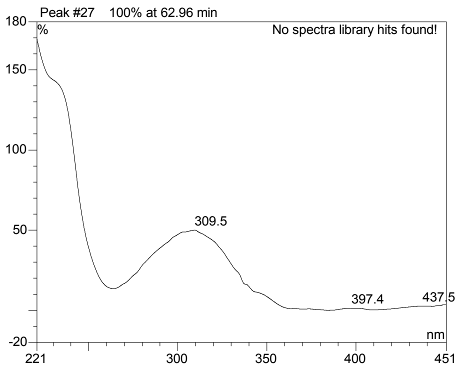


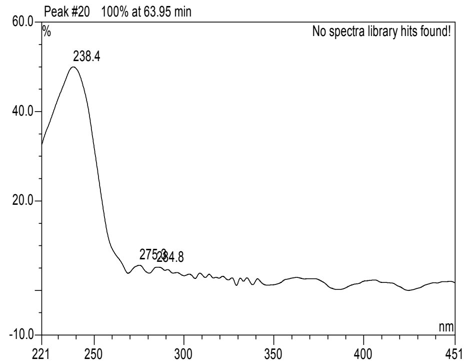
62.96 -

NI 137

63.58 -

NI 138





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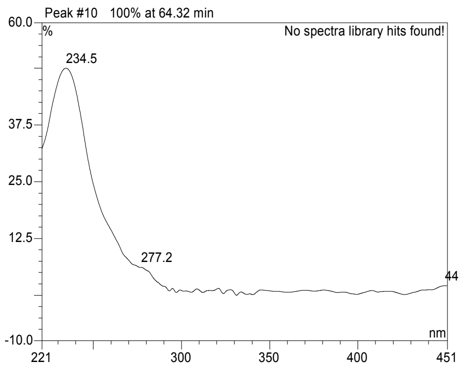
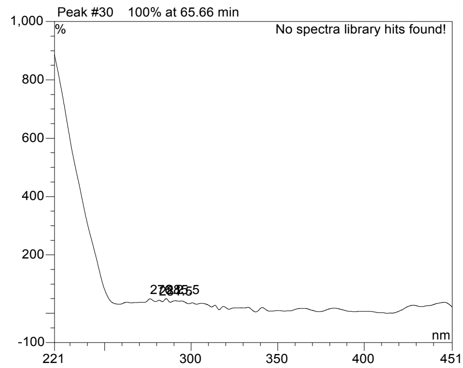
NI 139

65.66 -

NI 140





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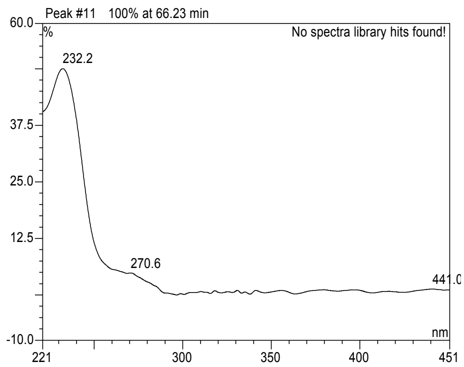
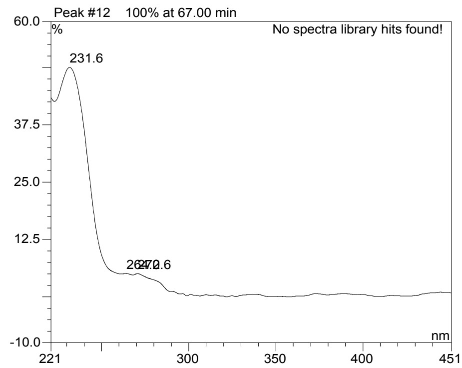
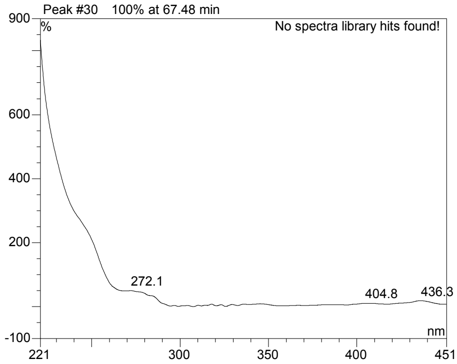
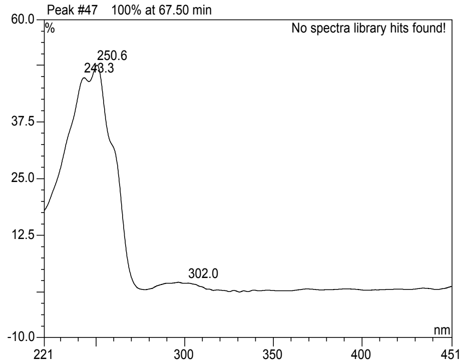
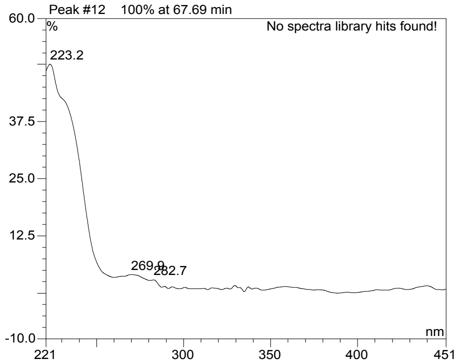
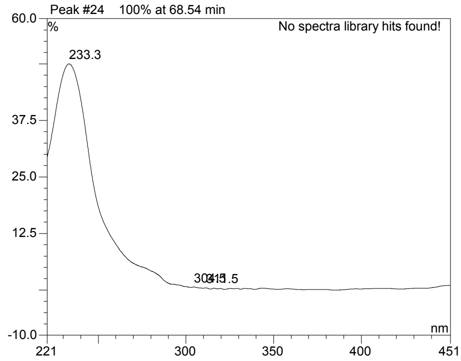
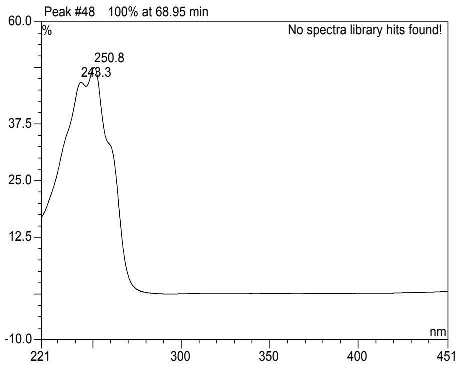
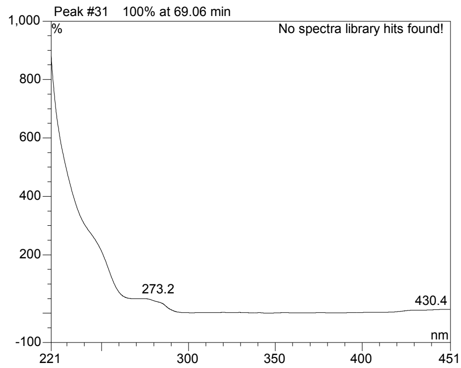
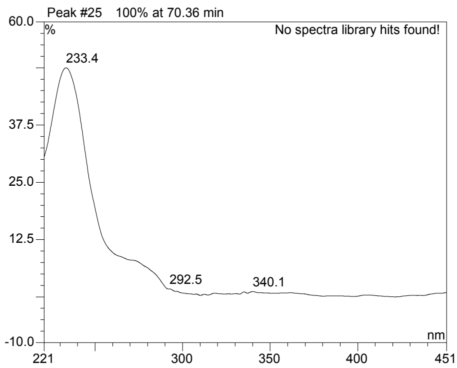
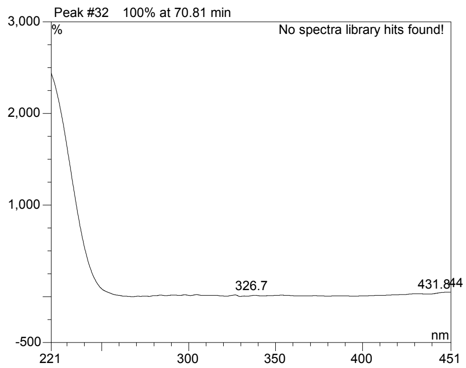
NI 141

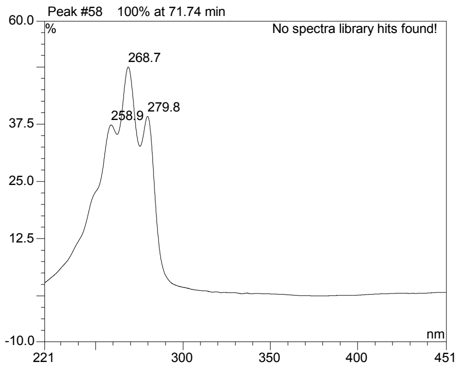
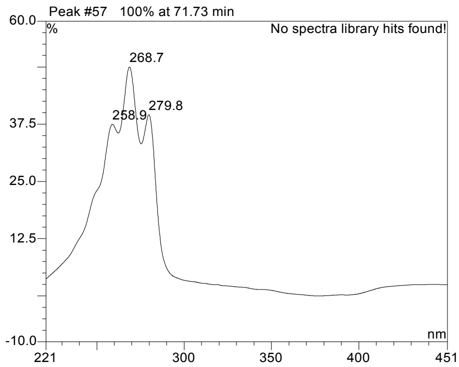
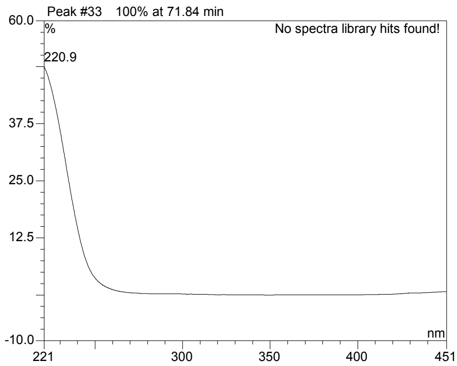
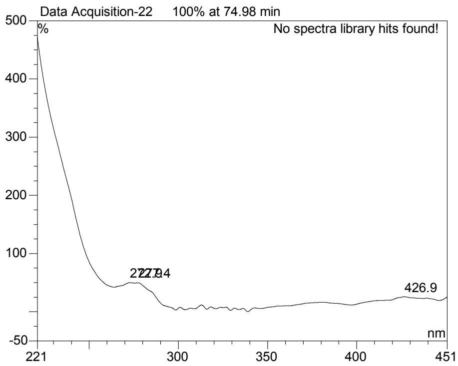
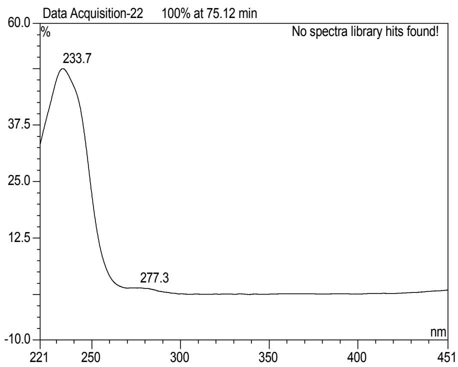
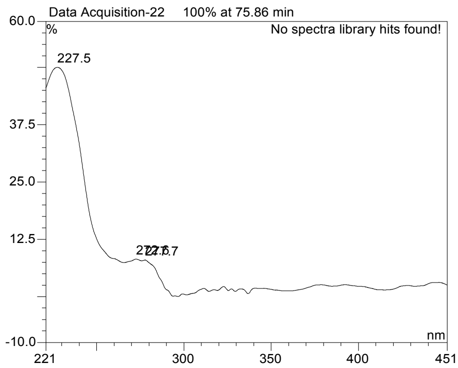
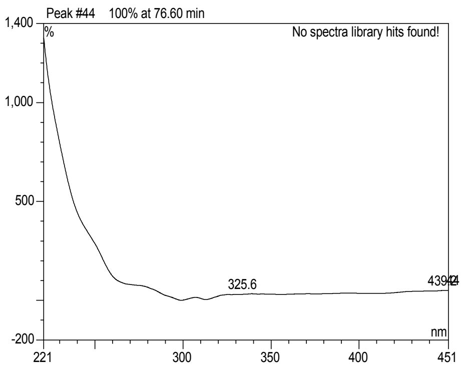
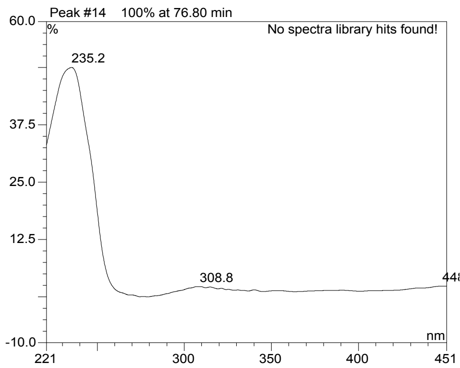
67.00 -

NI 142





67.48 - **NI 143** 67.50 - **NI 144**		
67.69 - **NI 145** 68.54 - **NI 146**		
68.95 - **NI 147** 69.06 - **NI 148**		
70.36 - **NI 149** 70.81 - **NI 150**		

71.44 - **NI 151** 71.73 - **NI 152**		
71.84 - **NI 153** 74.98 - **NI 154**		
75.12 - **NI 155** 75.83 - **NI 156**		
76.60 - **NI 157** 76.80 - **NI 158**		

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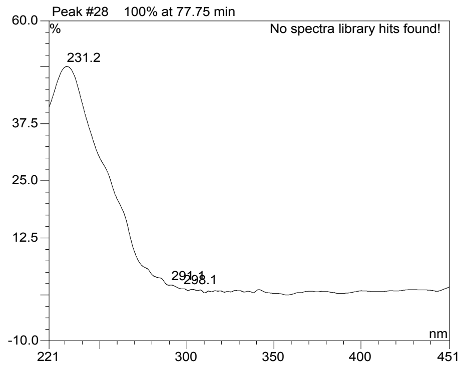
<td>
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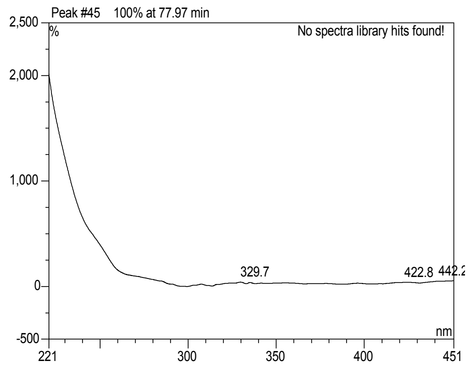
<td>
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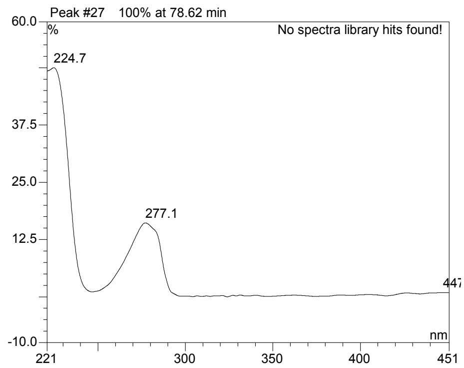
<td>
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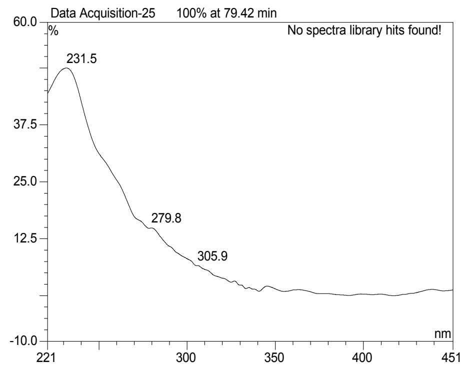
<td>
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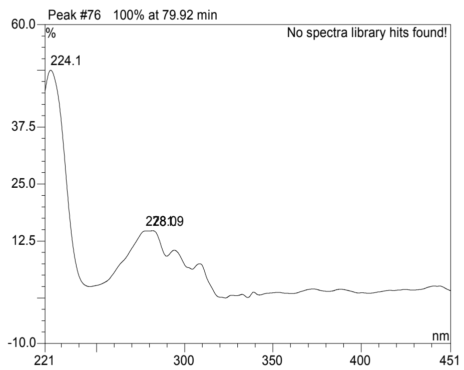
<td>
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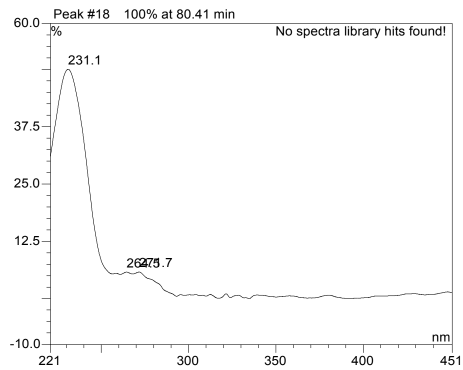
<td>
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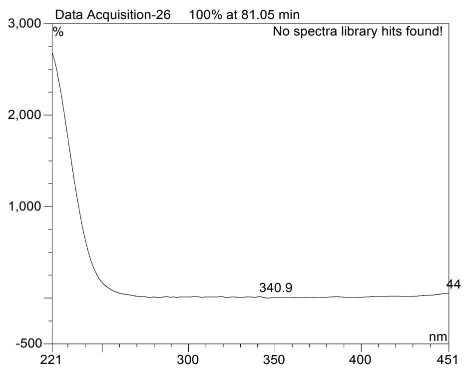
NI 166</td>
<td>
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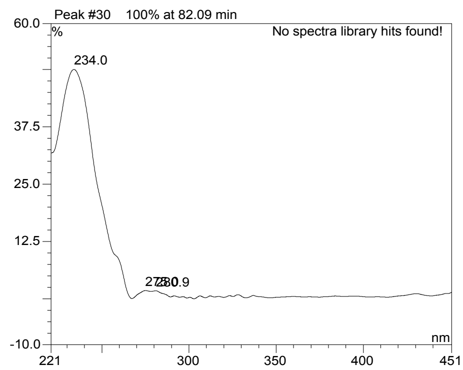
<td>
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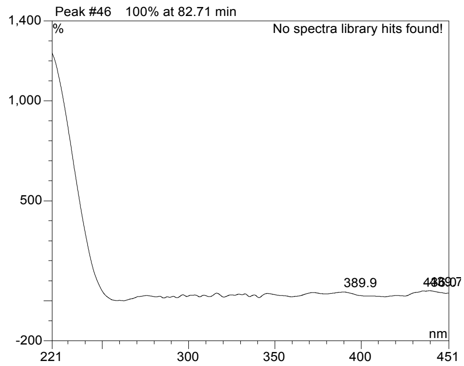
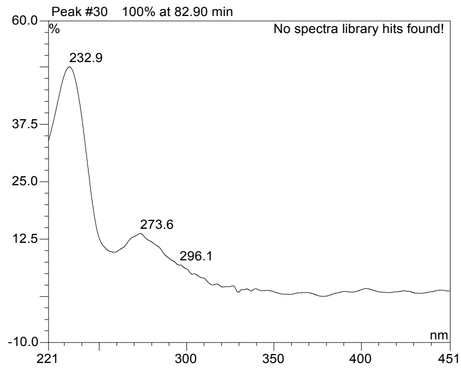
NI 168</td><td>
</td><td>
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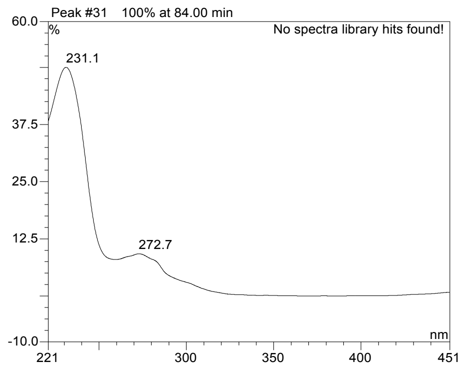
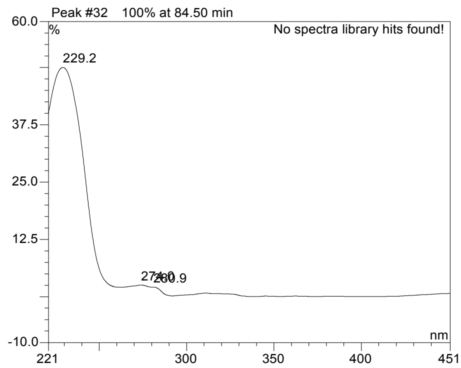
NI 170</td><td>
</td><td>
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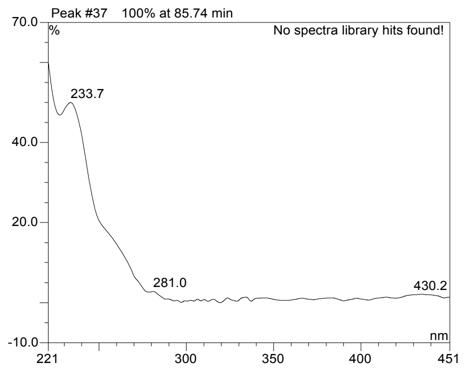
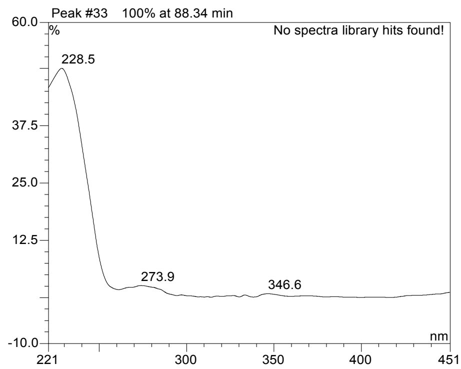
NI 172</td><td>
</td><td>
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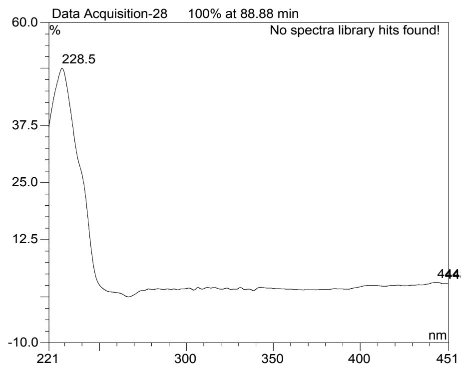
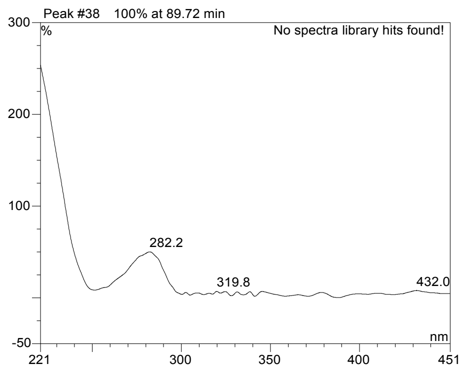
NI 174</td><td>
</td><td>
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90.84 -
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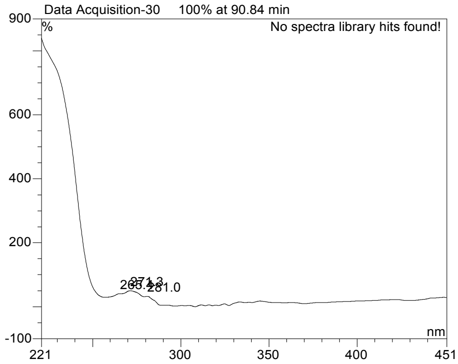
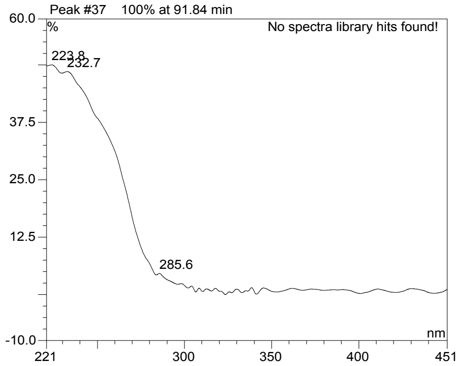
91.84 -
NI 176





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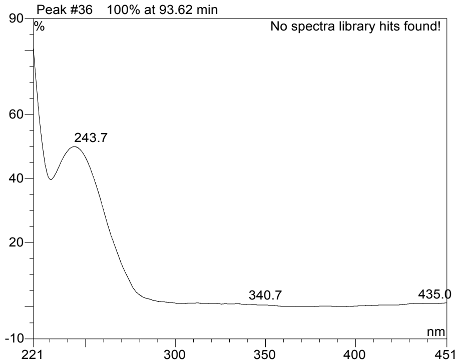
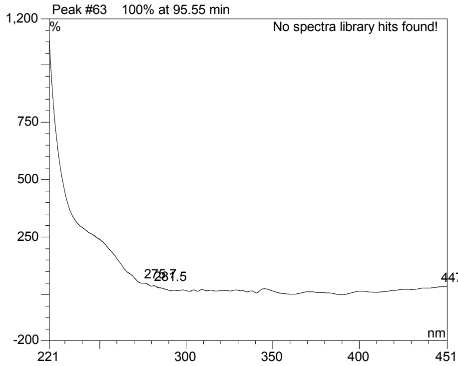
NI 178





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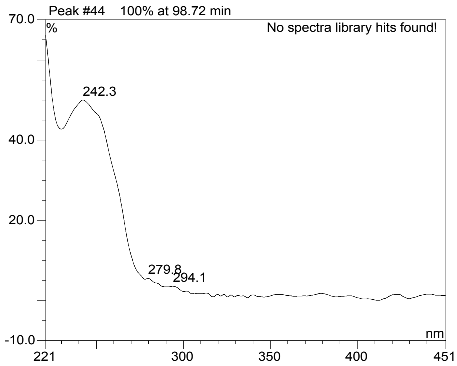
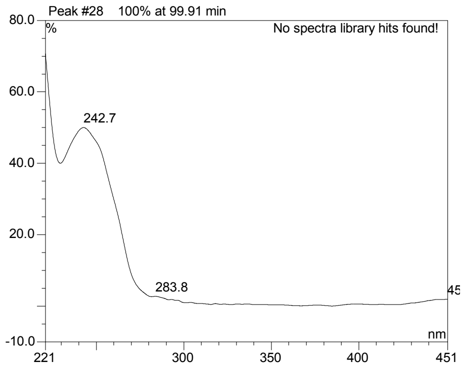
NI 181





101.40 -
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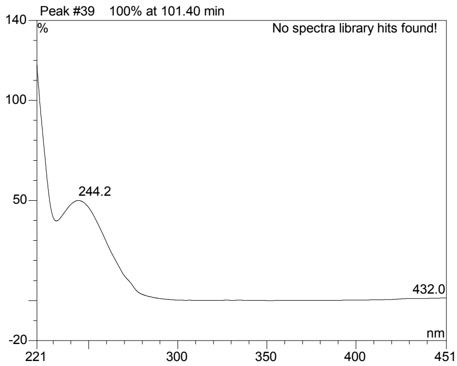
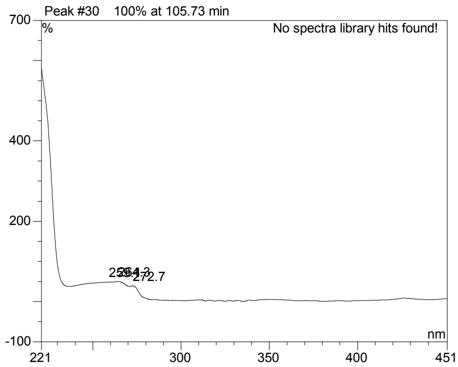
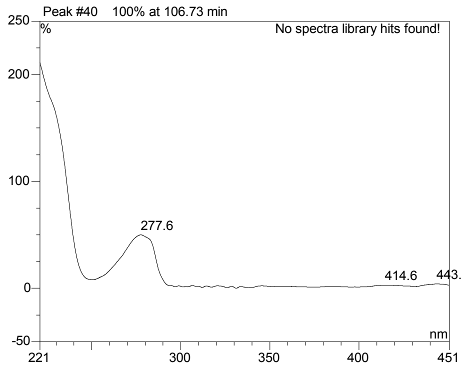
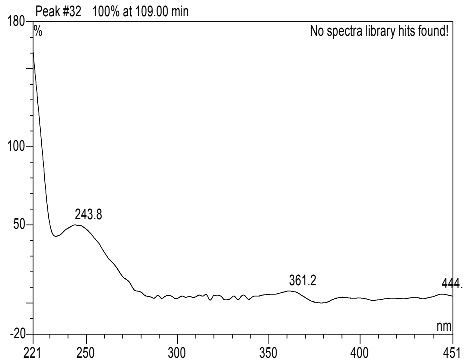
105.73 -
NI 183





106.73 - **NI 184** 109.00 - **NI 185**		

- **6.3. UV Spectra Experiment 3**

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a)</th><th>UV
b)</th></tr>
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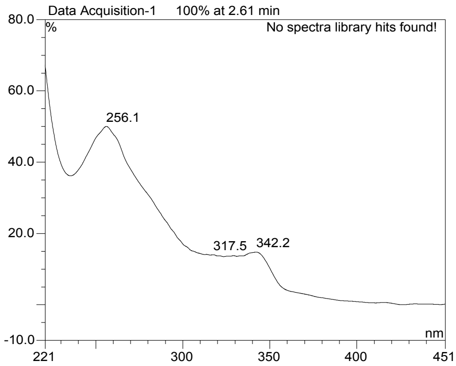
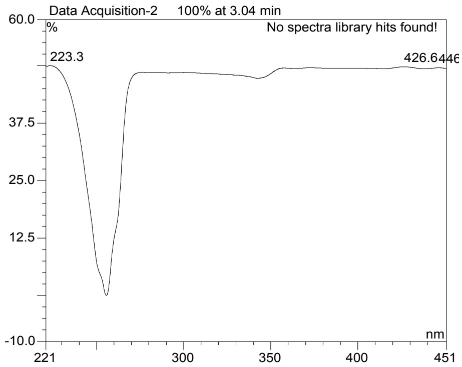
NI 1
3.04 -
NI 2</td><td>
</td><td>
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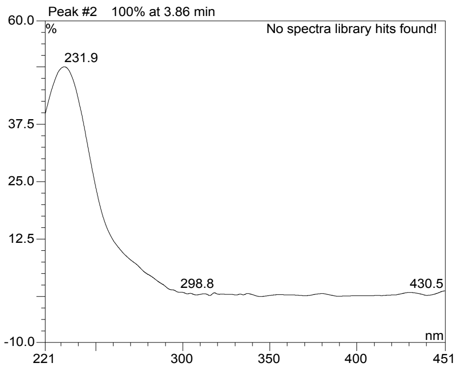
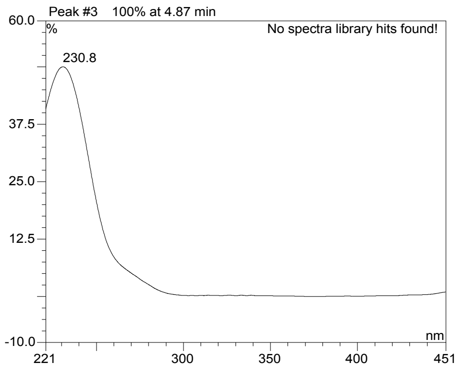
NI 3
4.87 -
NI 4</td><td>
</td><td>
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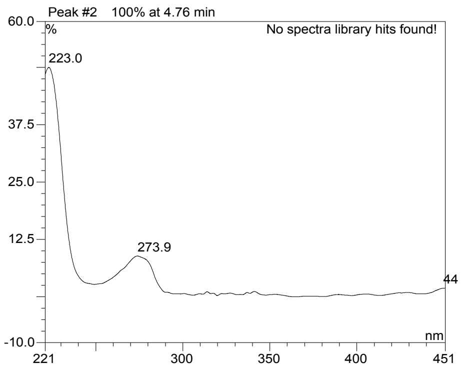
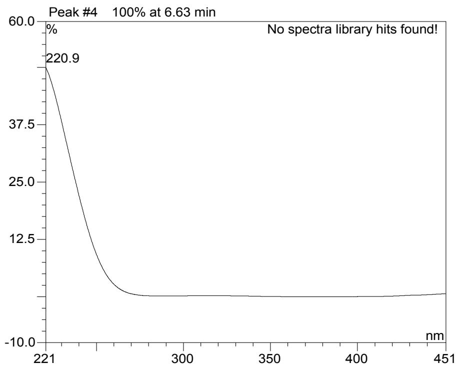
NI 5
6.63 -
NI 6</td><td>
</td><td>
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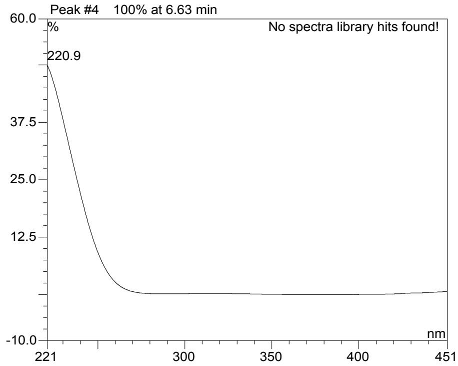
NI 8</td>
<td>
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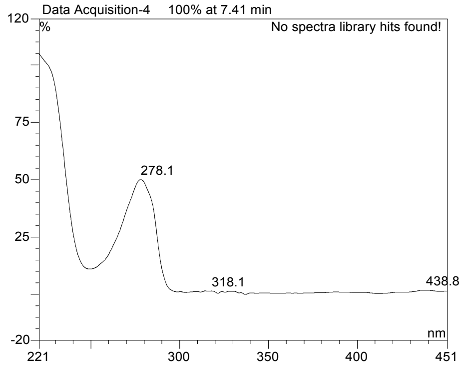
<td>
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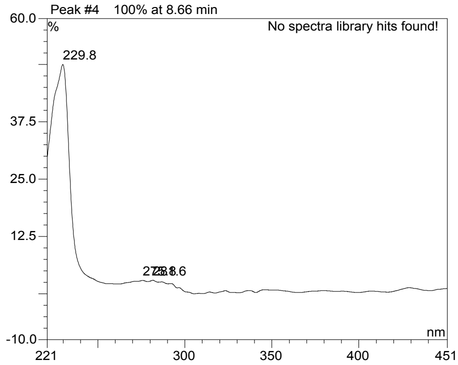
NI 9
9.59 -
NI 10</td>
<td>
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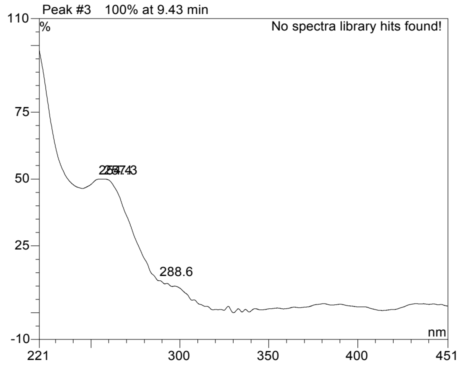
<td>
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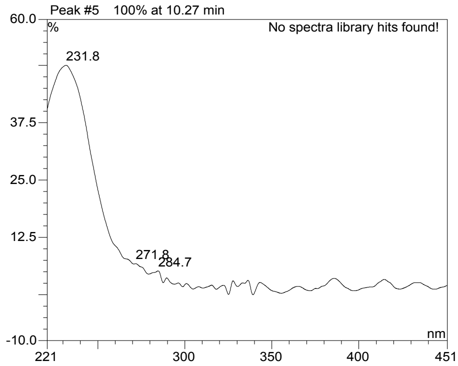
NI 12</td>
<td>
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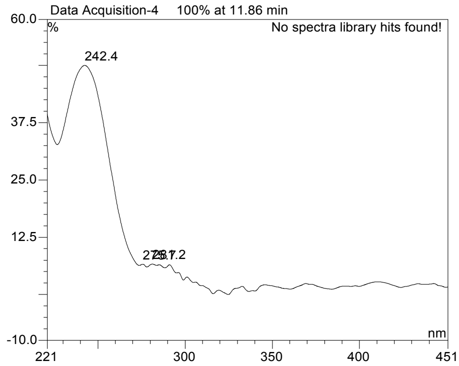
<td>
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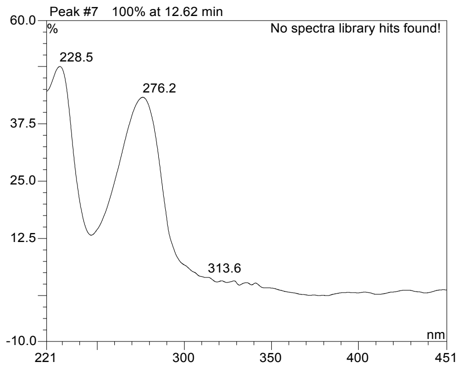
13.47 -
NI 14</td>
<td>
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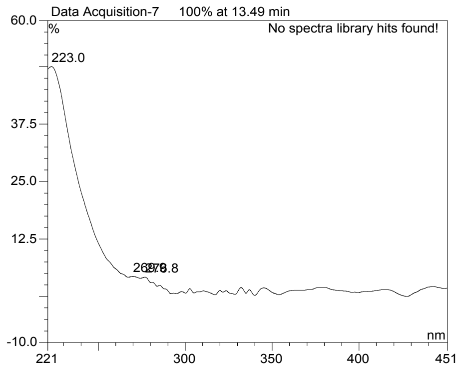
<td>
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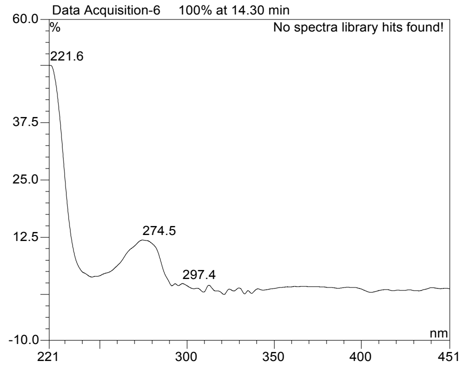
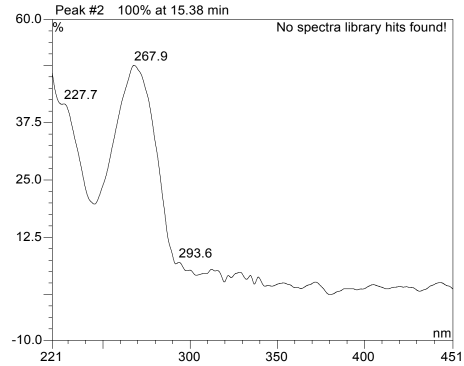
14.30 -
NI 15
15.18 -
NI 16





17.31 -
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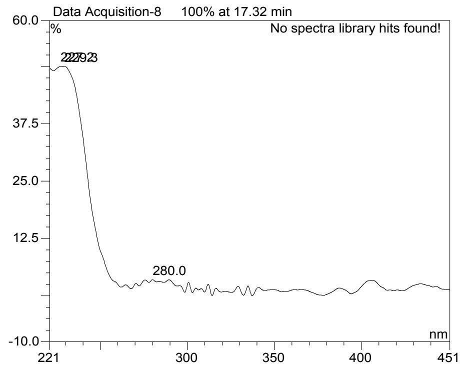
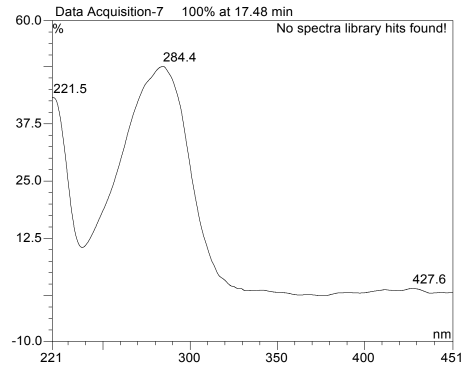
NI 18





18.87 -
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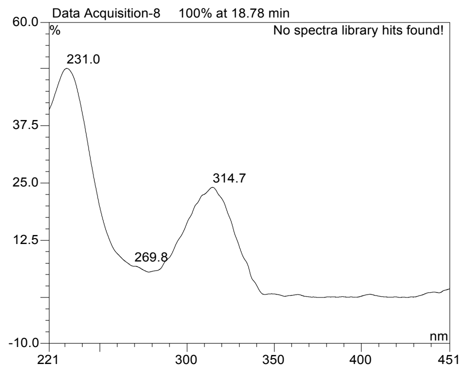
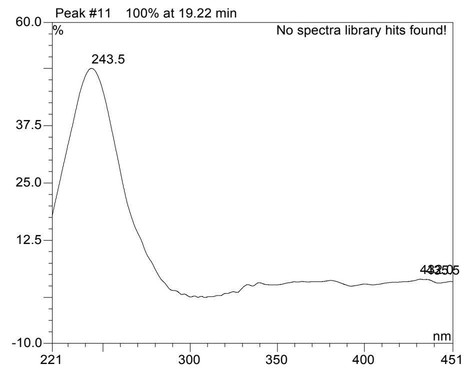
NI 20





19.82 -
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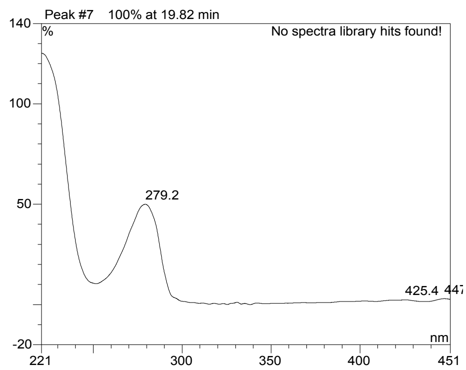
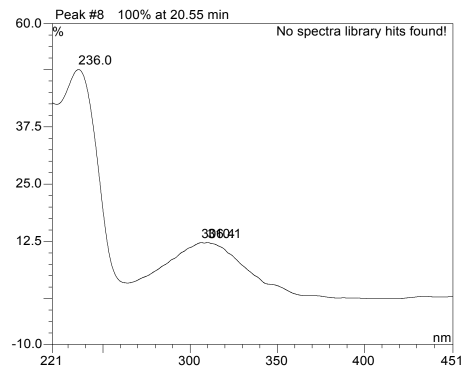
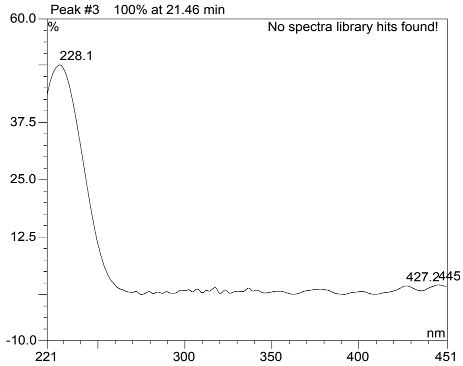
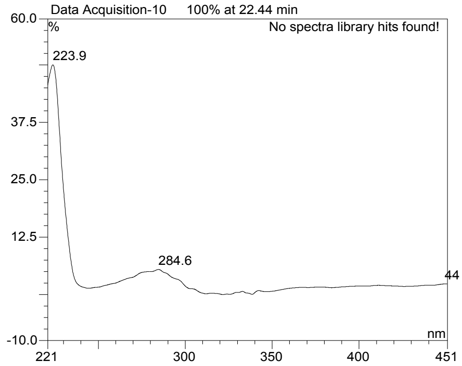
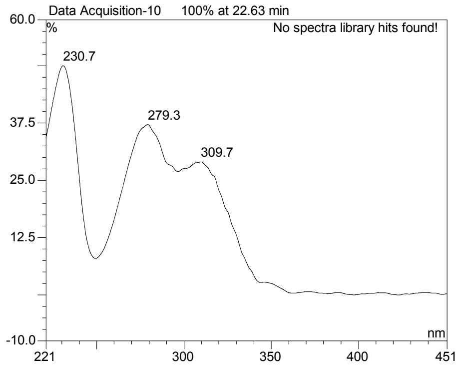
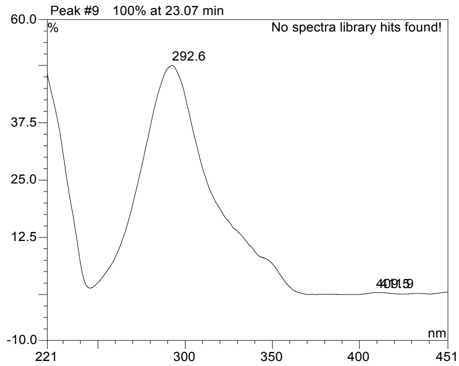
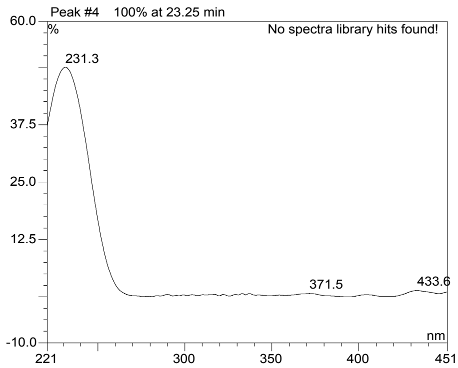
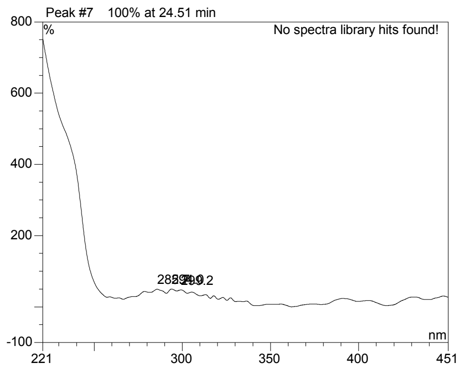
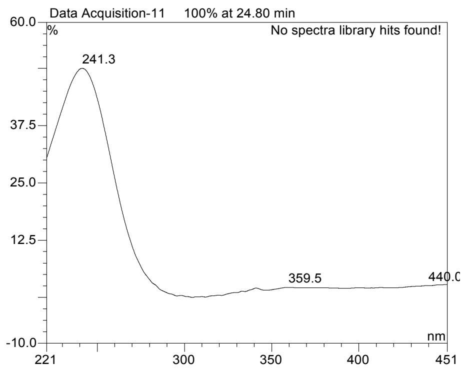
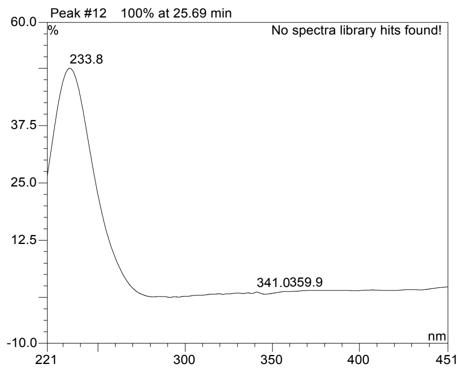
NI 22





21.46 - **NI 23** 22.44 - **NI 24**	 	
22.63 - **NI 25** 23.07 - **NI 26**	 	
23.25 - **NI 27** 24.51 - **NI 28**	 	
24.80 - **NI 29** 25.69 - **NI 30**	 	

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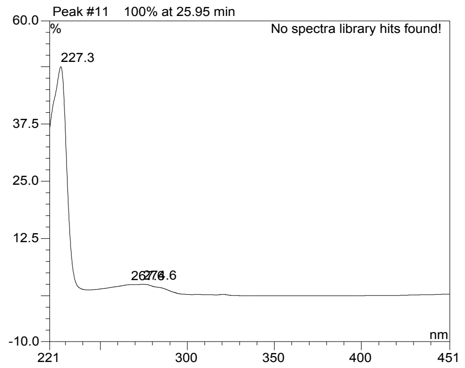
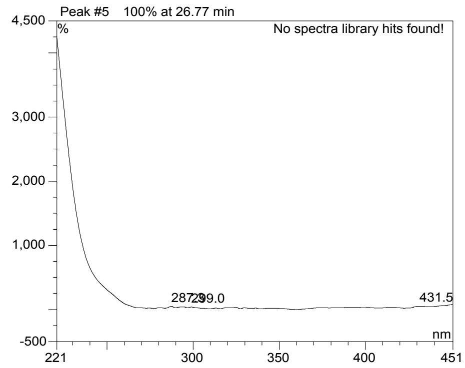
<td></td>
<td></td>
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28.30 -
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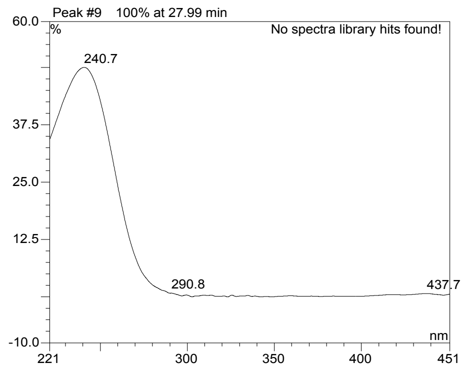
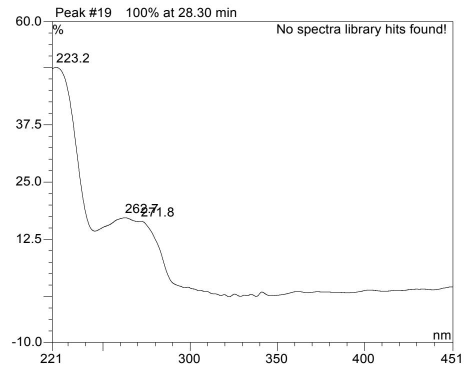
<td></td>
<td></td>
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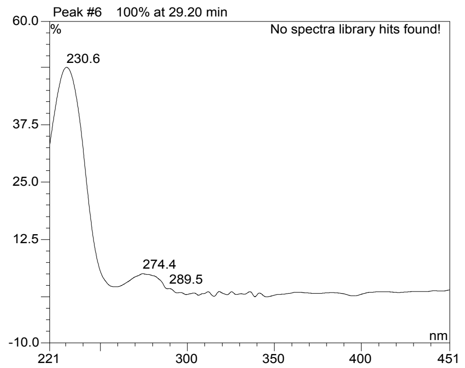
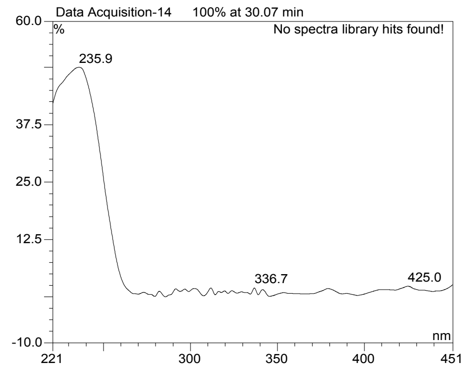
<td></td>
<td></td>
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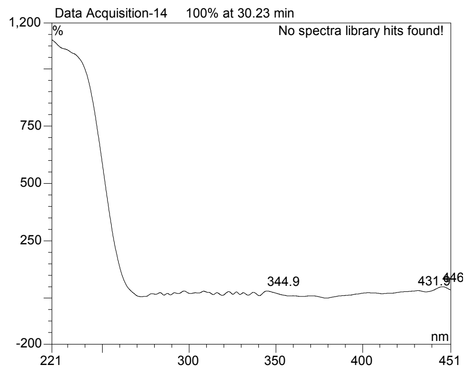
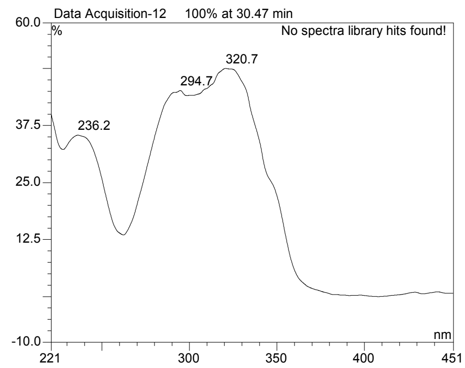
<td></td>
<td></td>
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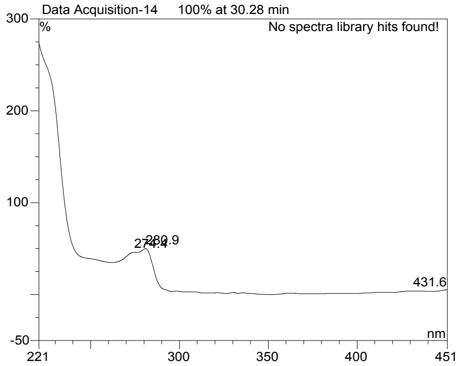
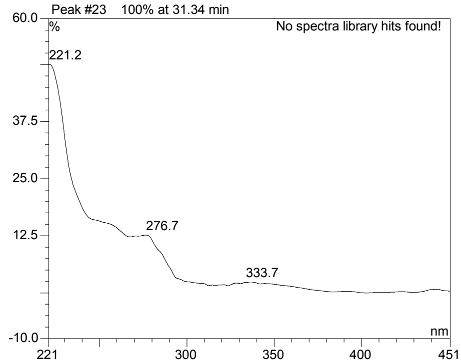
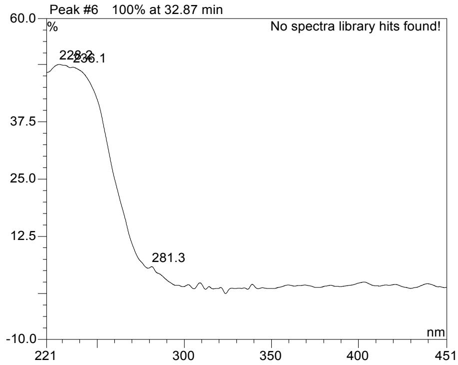
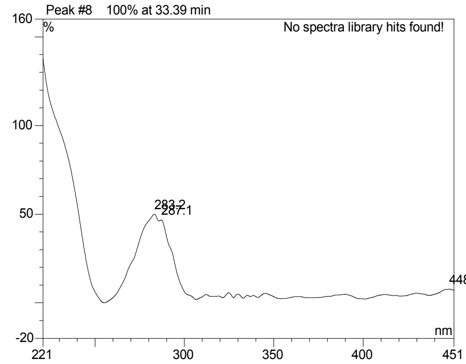
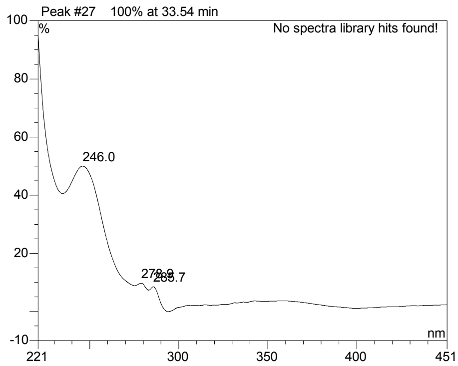
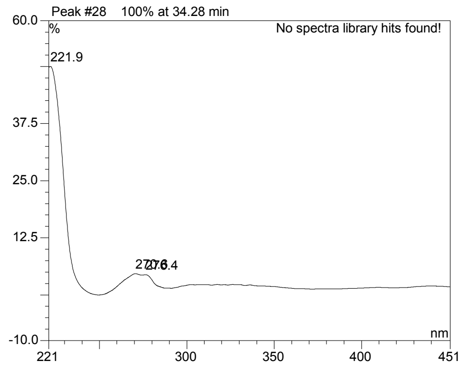
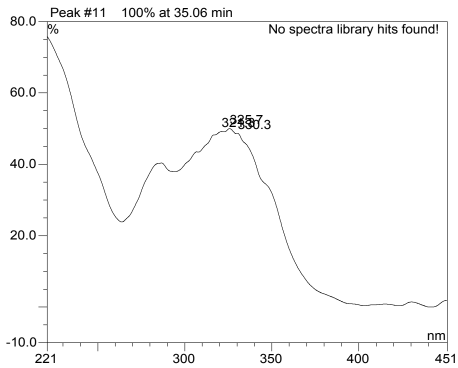
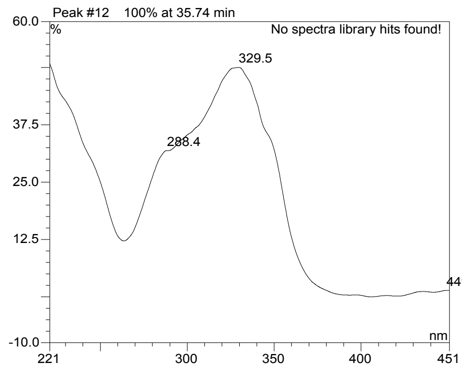
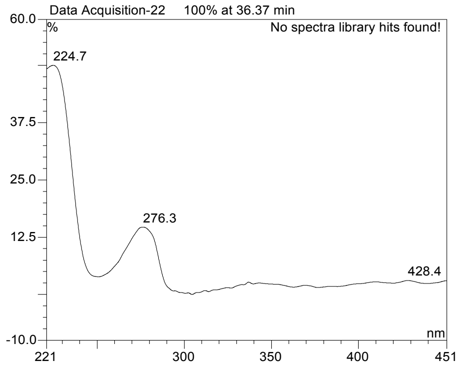
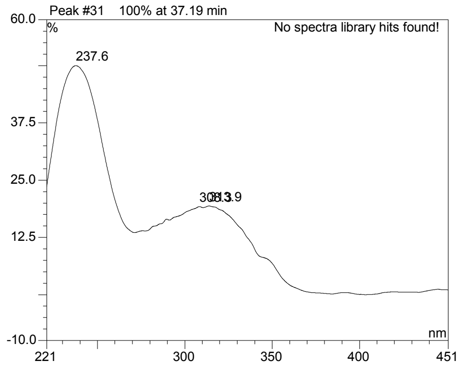
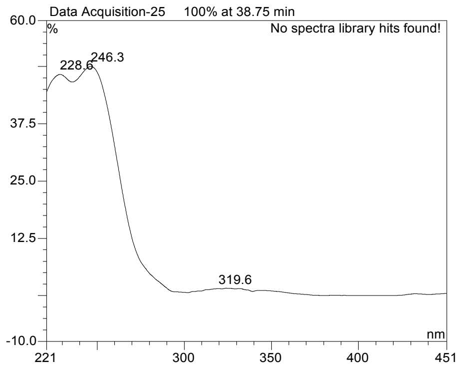
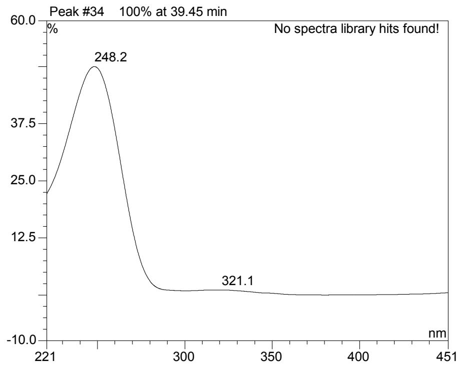
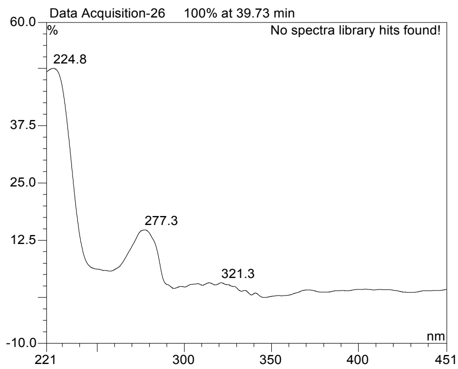
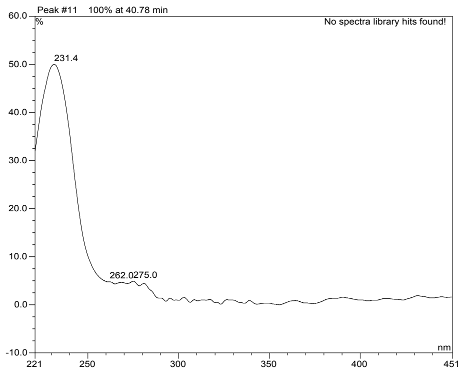
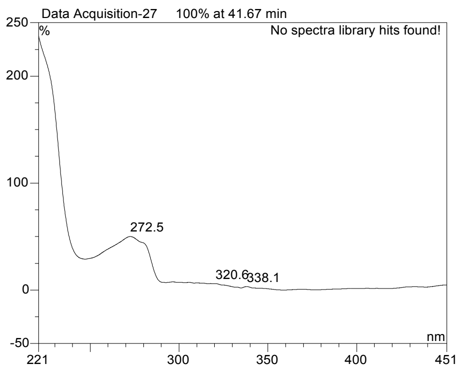
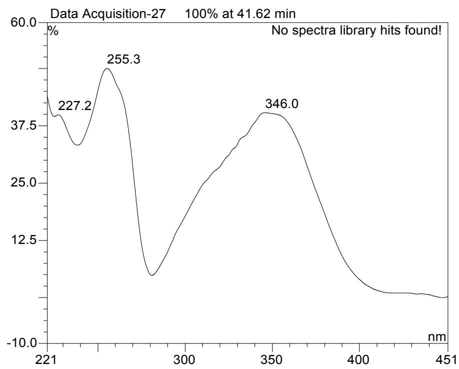
30.99 - **NI 37** 31.34 - **NI 38**		
32.66 - **NI 39** 33.39 - **NI 40**		
33.54 - **NI 41** 34.28 - **NI 42**		
35.06 - **Chlorogenic acid 1** 35.74 - **Chlorogenic acid 2**		

36.37 - **NI 43** 37.19 - **NI 44**		
38.53 - **NI 45** 39.45 - **NI 46**		
39.73 - **NI 47** 40.78 - **NI 48**		
41.54 - **NI 49** 41.62 - **Quercetin glycoside**		

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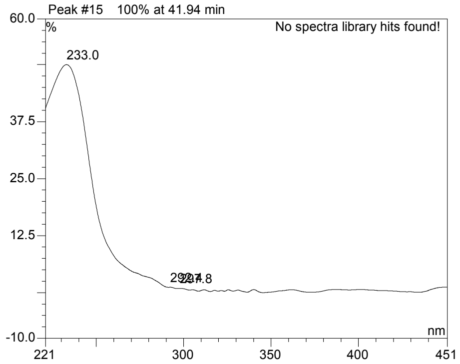
<td>
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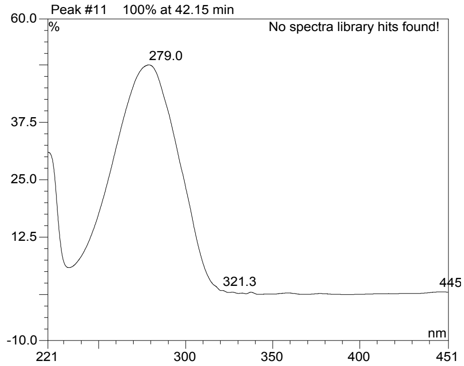
<td>
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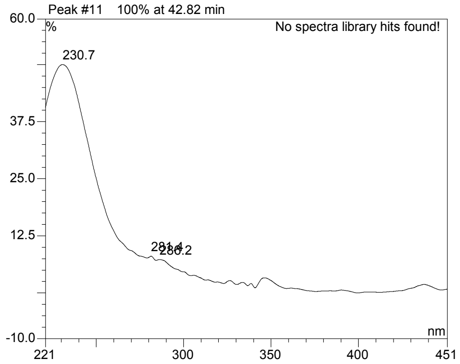
<td>
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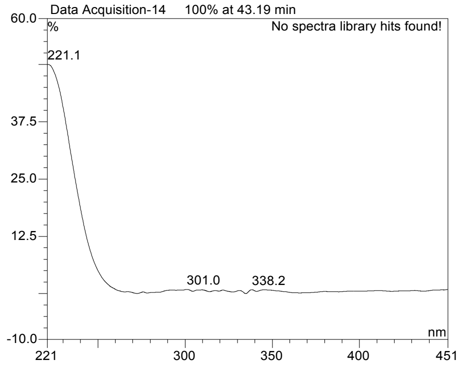
<td>
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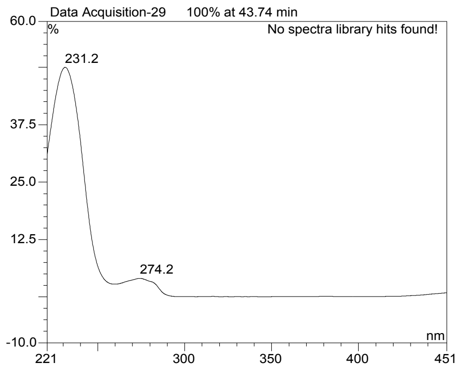
<td>
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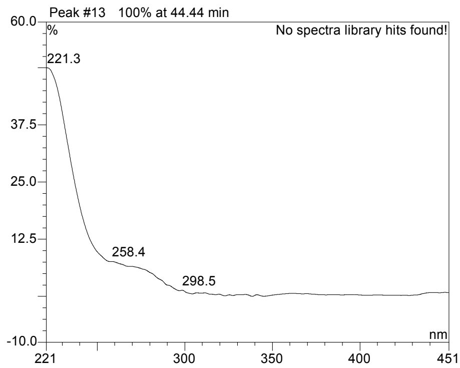
<td>
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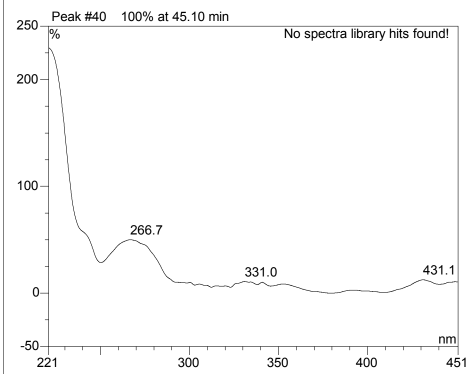
<td>
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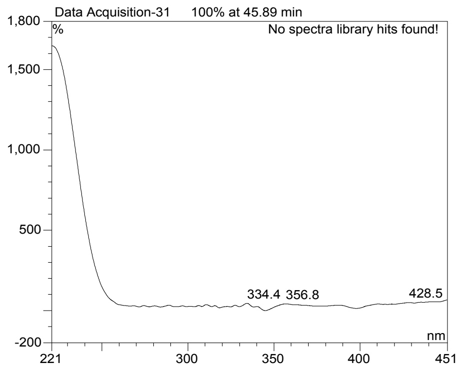
<td>
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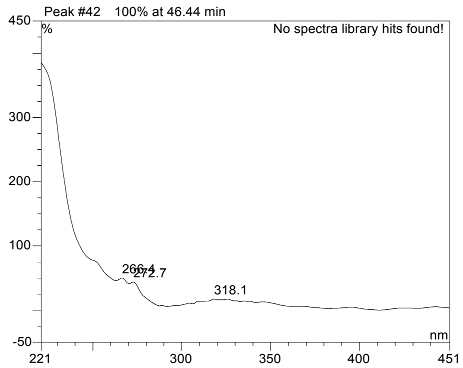
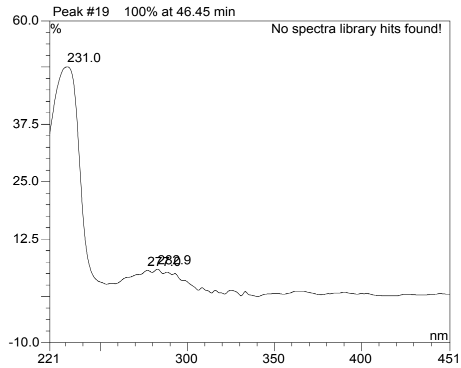
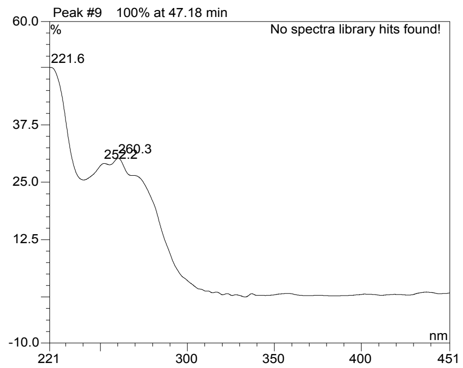
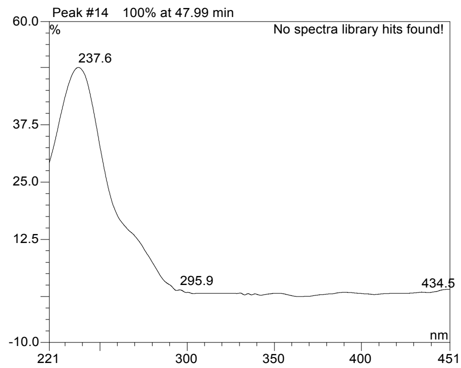
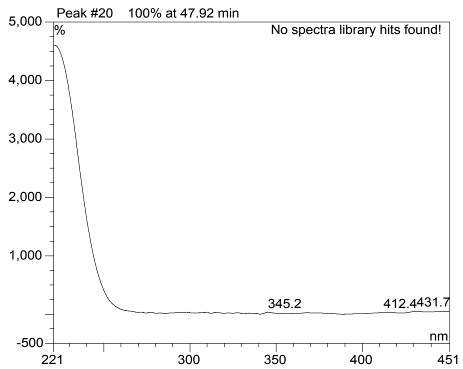
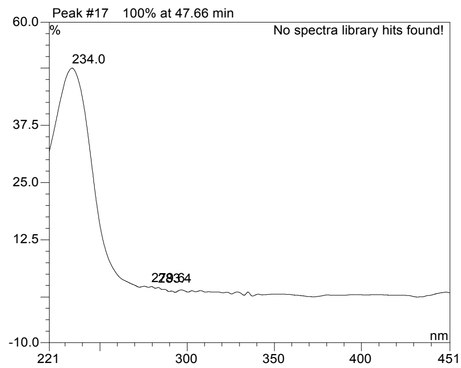
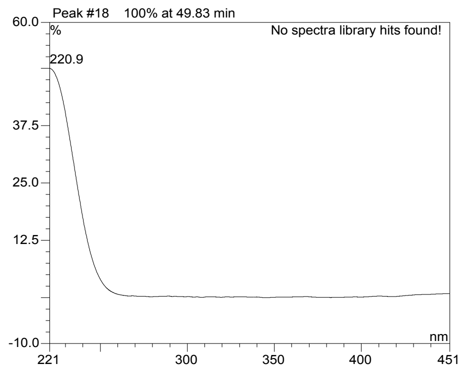
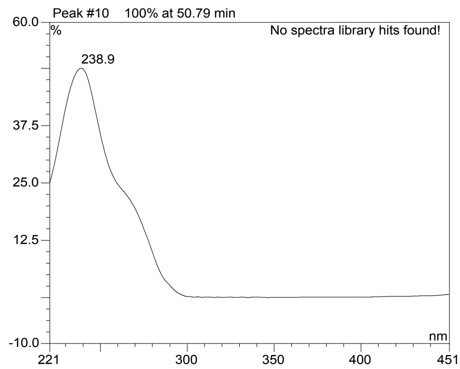
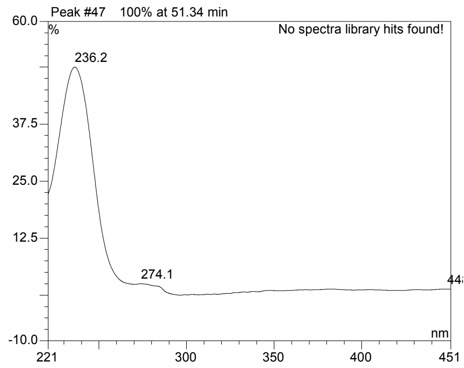
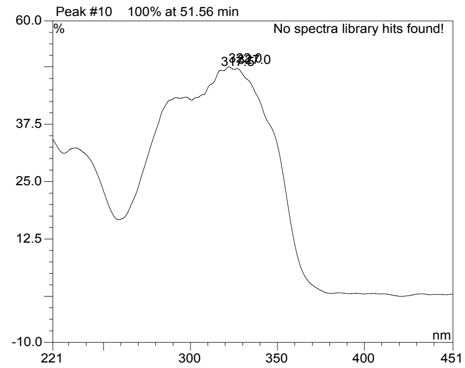
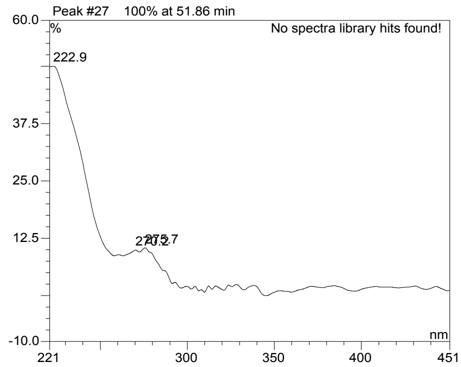
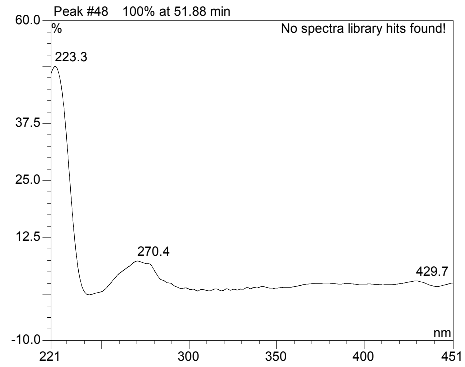
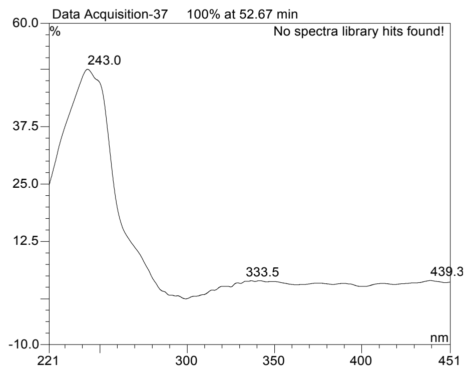
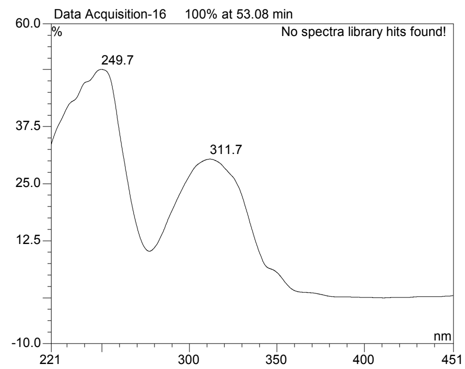
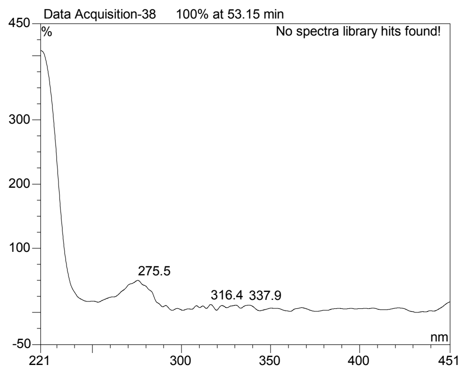
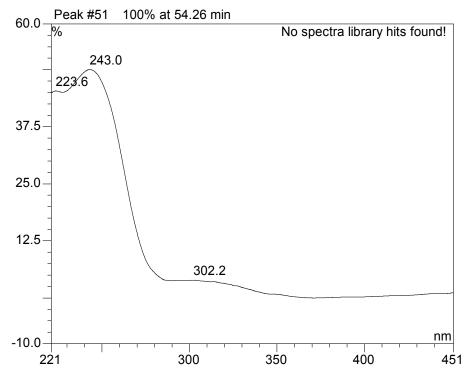
46.44 - **NI 57** 46.45 - **NI 58**		
47.18 - **NI 59** 47.92 - **NI 60**		
47.92 - **NI 61** 47.96 - **NI 62**		
49.83 - **NI 63** 50.79 - **NI 64**		

51.34 - **NI 65** 51.56 - **Unknown cinnamic acid derivative**		
51.87 - **NI 66** 51.88 - **NI 67**		
52.85 - **NI 68** 53.08 - **NI 69**		
53.15 - **NI 70** 54.26 - **NI 71**		

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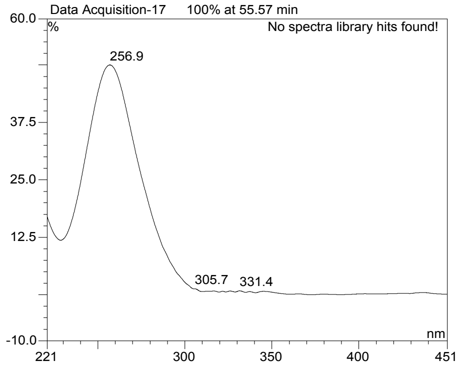
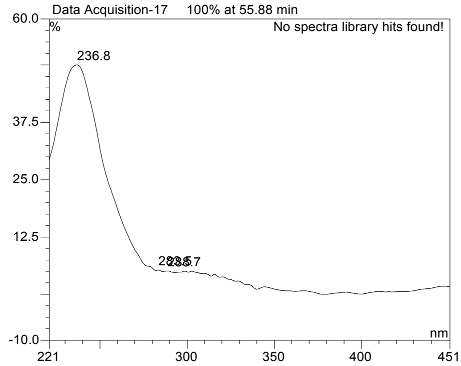
NI 72</td><td>
</td><td>
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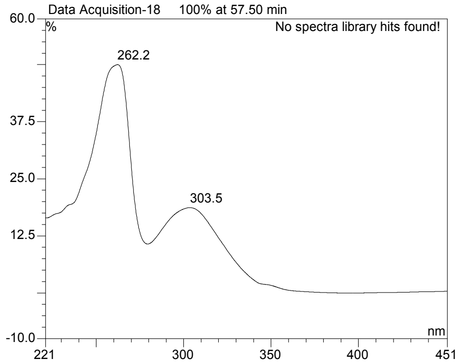
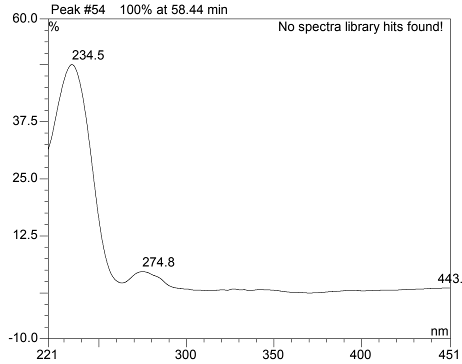
NI 73</td><td>
</td><td>
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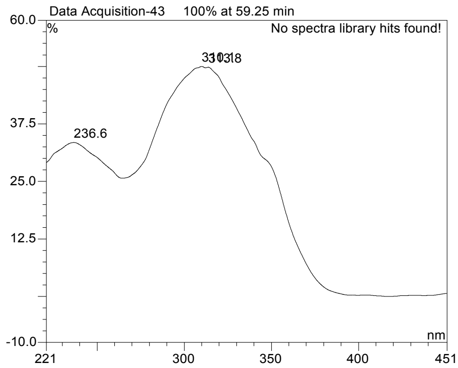
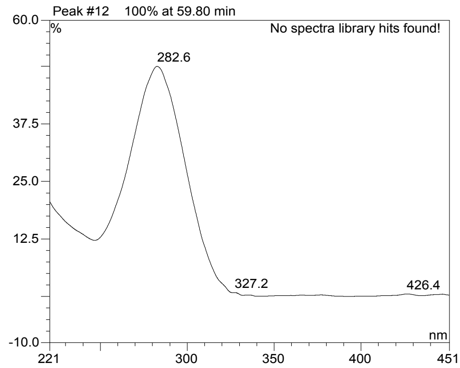
NI 75</td><td>
</td><td>
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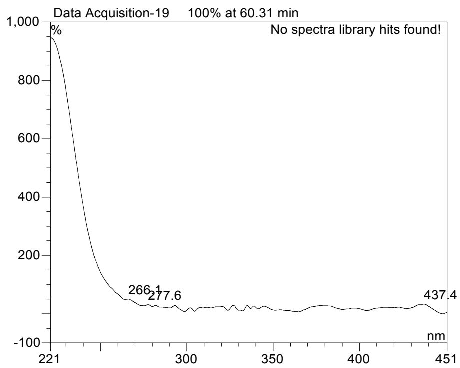
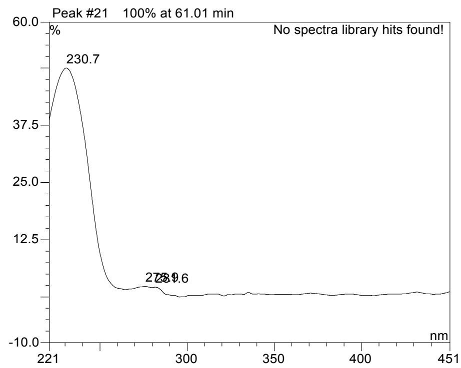
NI 77</td><td>
</td><td>
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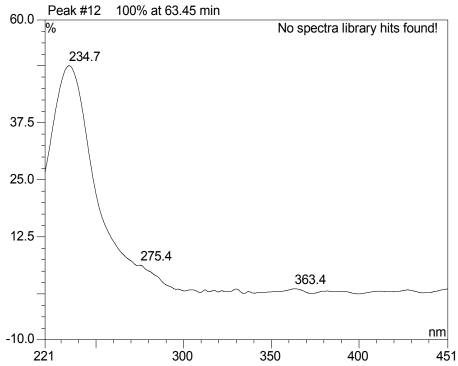
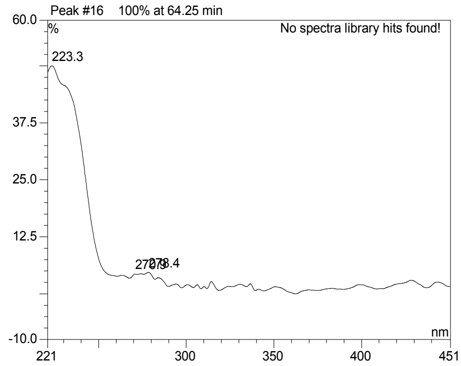
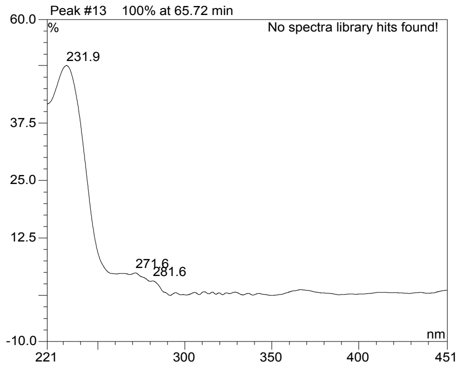
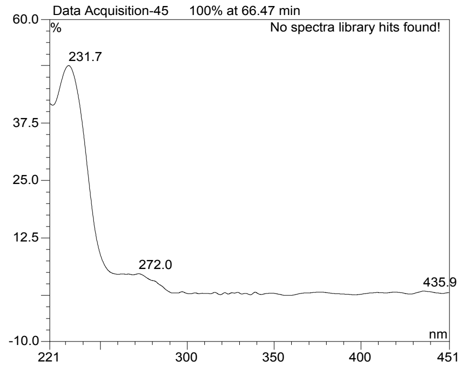
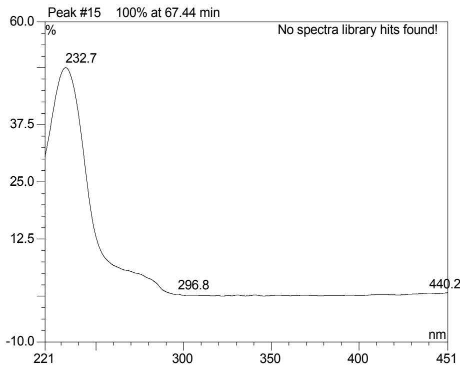
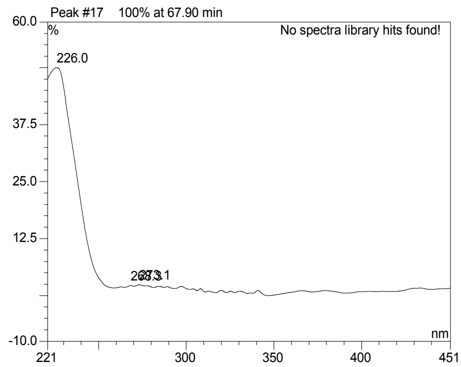
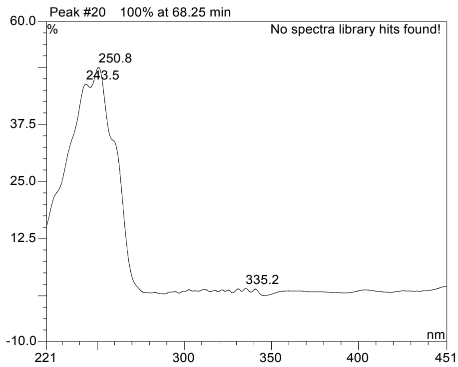
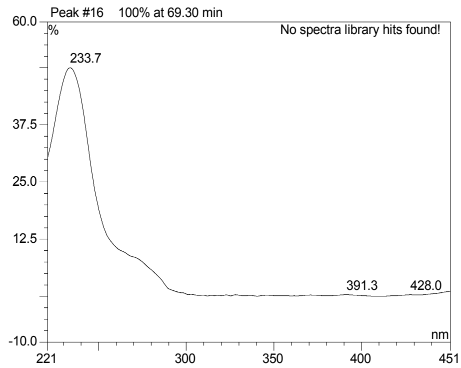
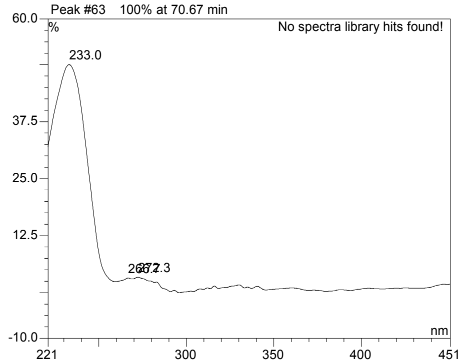
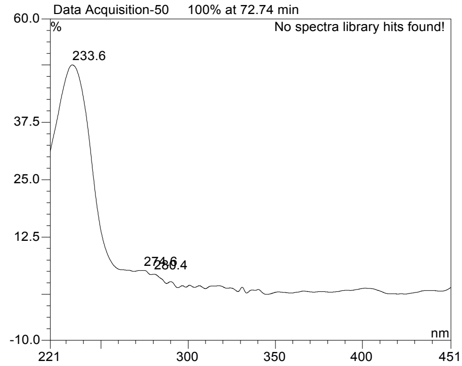
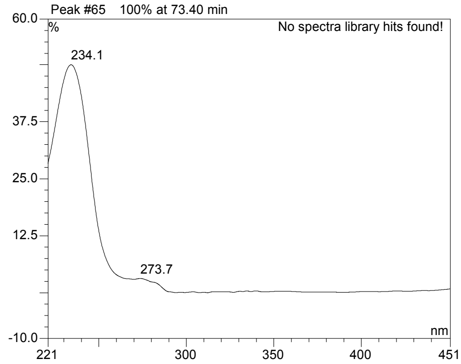
63.45 - **NI 78** 64.25 - **NI 79**		
65.72 - **NI 80** 66.47 - **NI 81**		
67.44 - **NI 82** 67.90 - **NI 83**		
68.25 - **NI 84** 69.30 - **NI 85**		

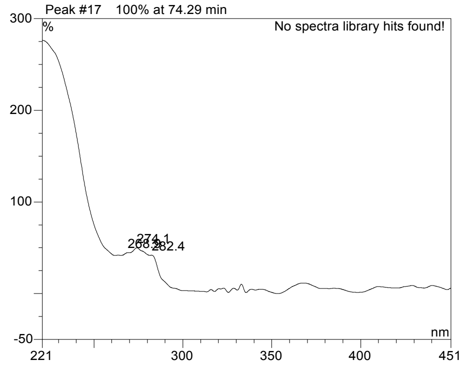
70.67 -
NI 86
72.49 -
NI 87





73.40 -
NI 88
74.26 -
NI 89





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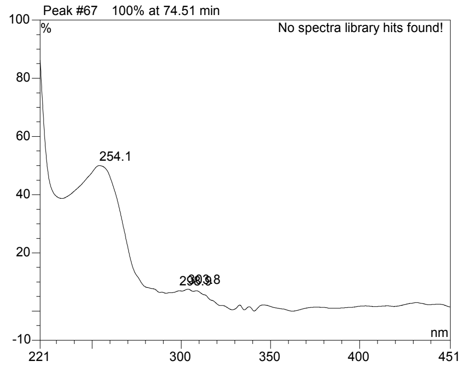
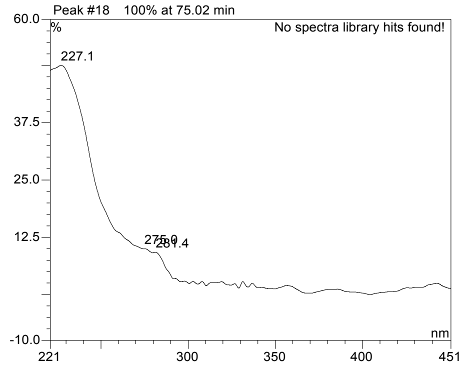
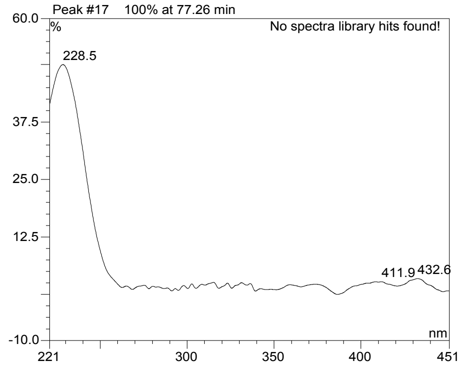
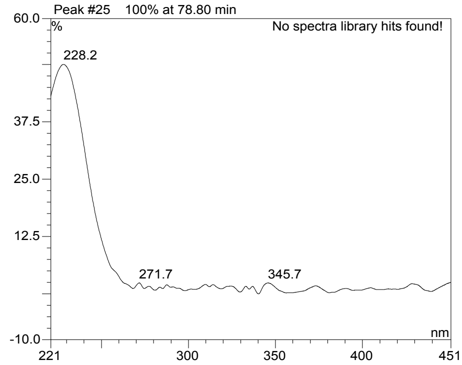
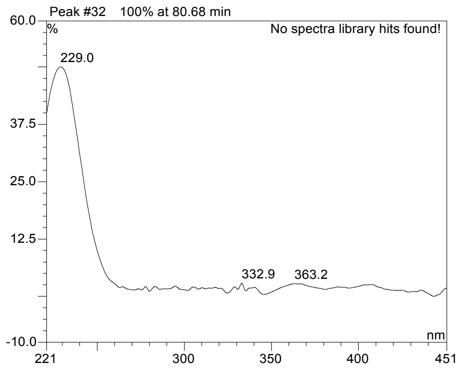
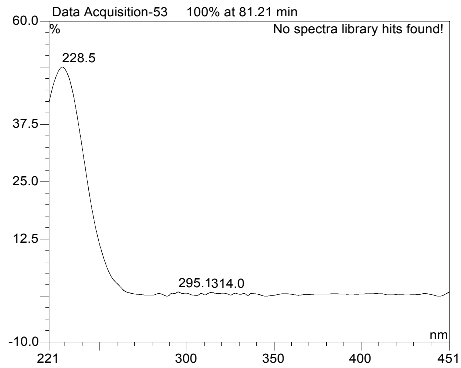
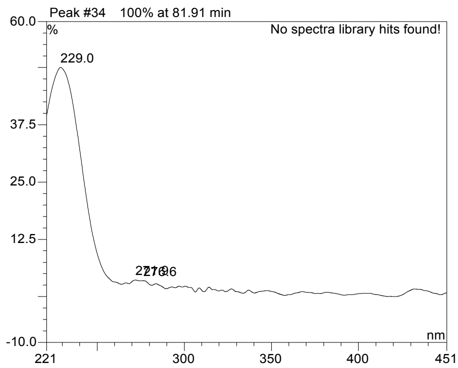
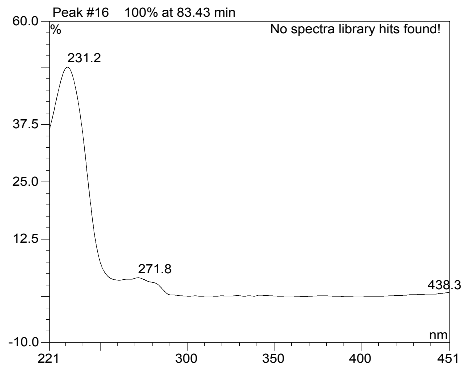
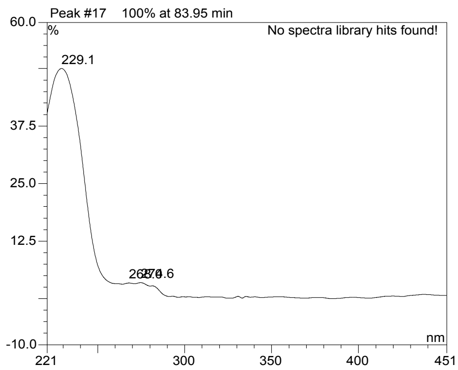
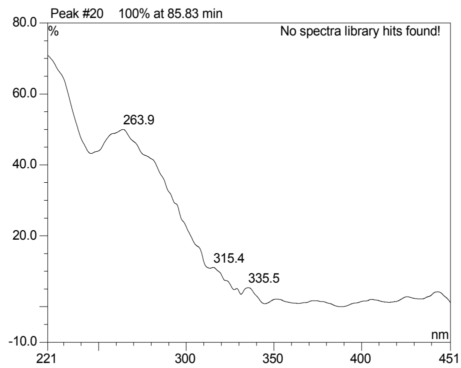
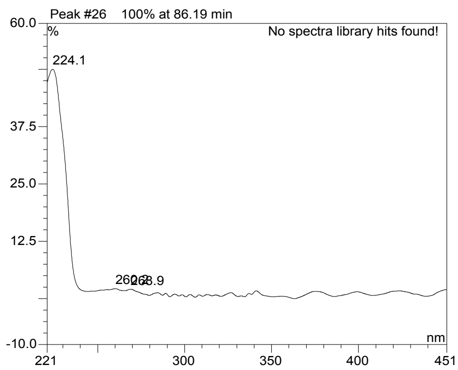
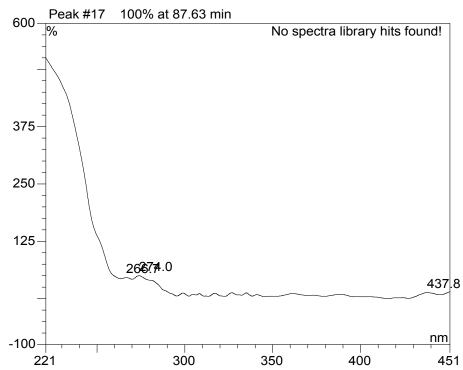




77.26 -
NI 92
78.80 -
NI 93





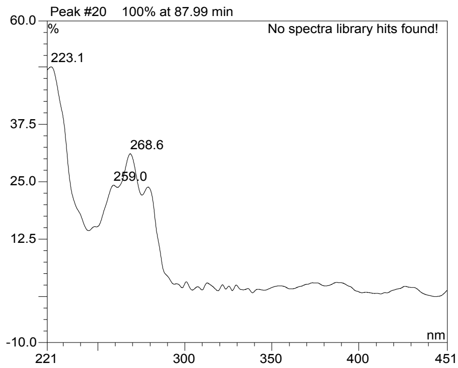
80.68 - **NI 94** 81.21 - **NI 95**		
81.91 - **NI 96** 83.43 - **NI 97**		
83.95 - **NI 98** 85.83 - **NI 99**		
86.19 - **NI 100** 87.81 - **NI 101**		

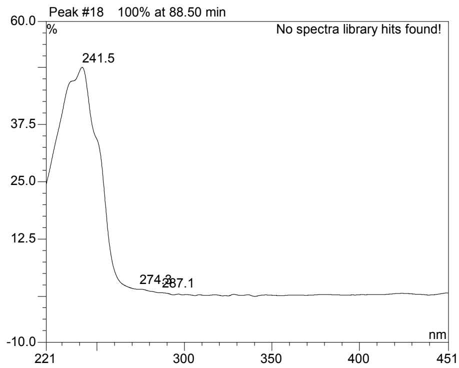
87.99 -

Poly-acetylene

88.50 -

NI 102



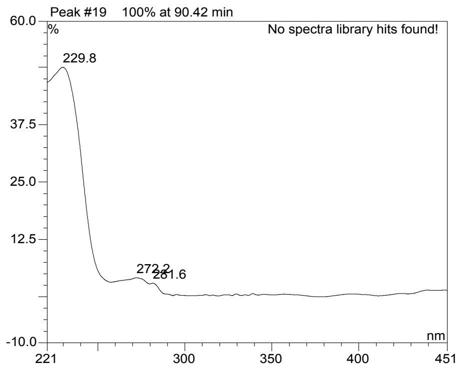


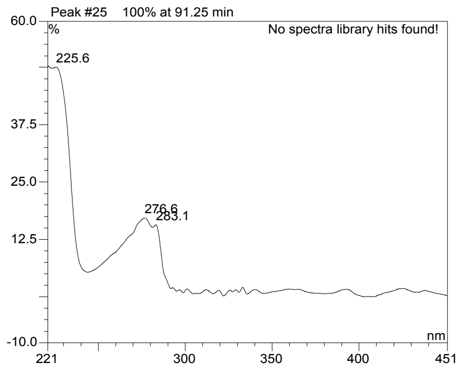
90.42 -

NI 103

91.25 -

NI 104



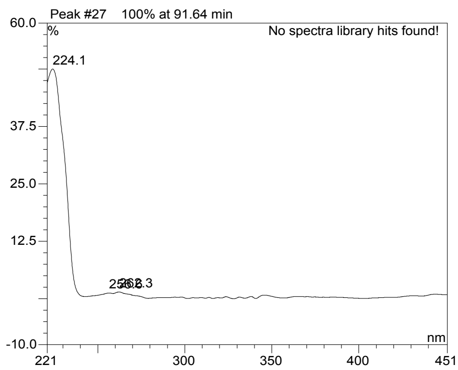


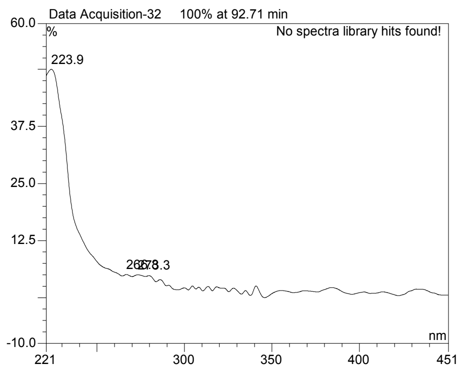
91.64 -

NI 105

92.71 -

NI 106



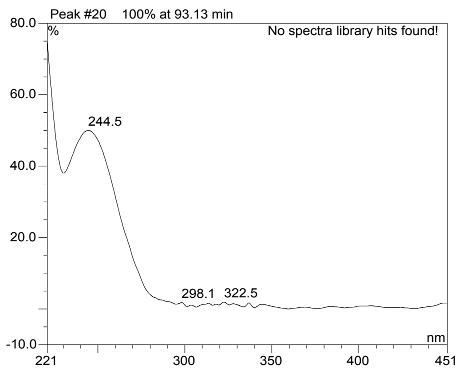


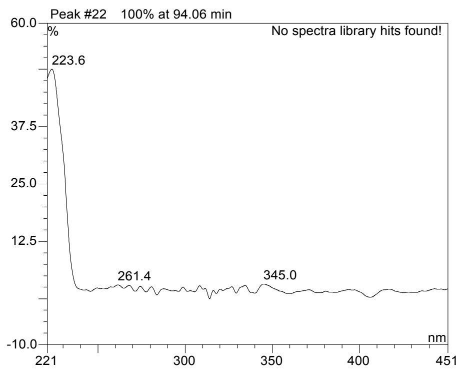
93.13 -

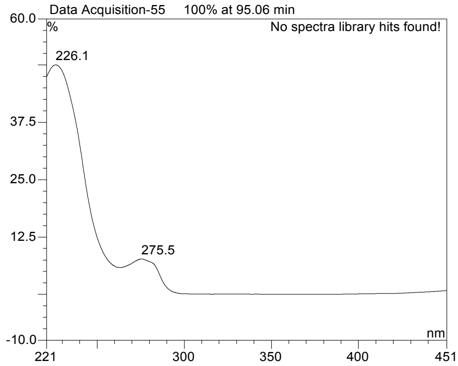
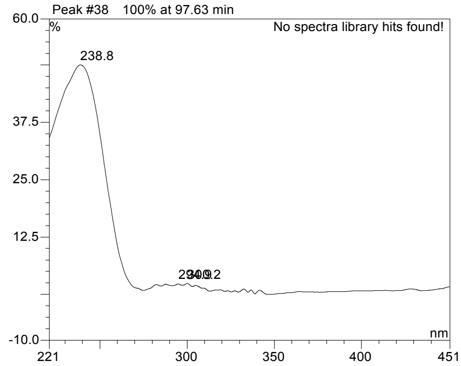
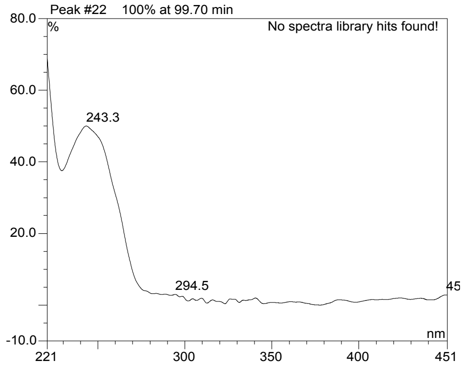
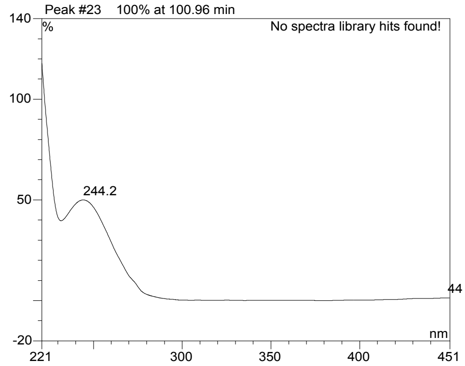
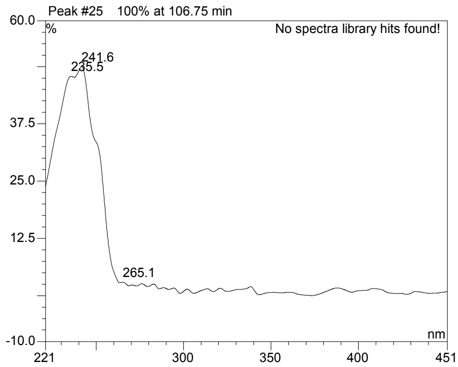
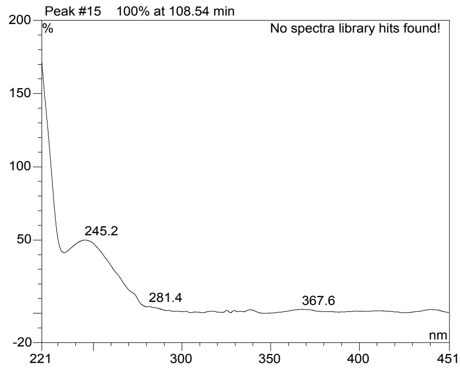
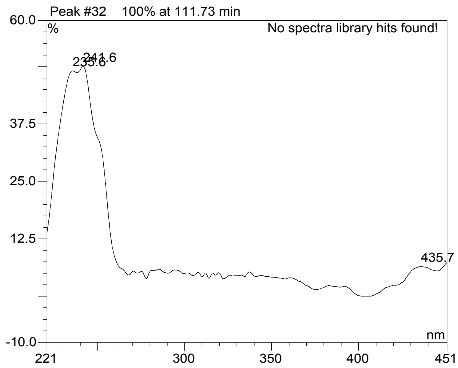
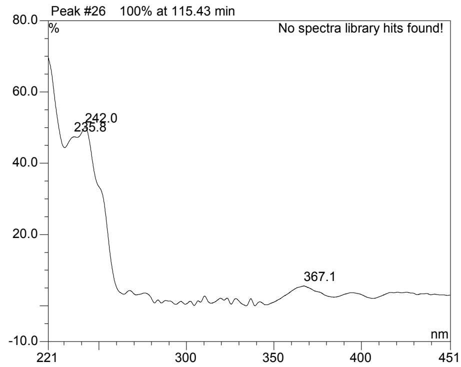
NI 107

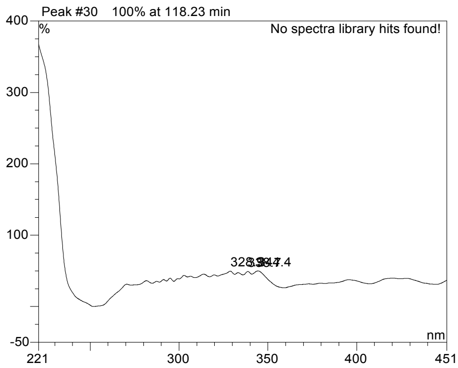
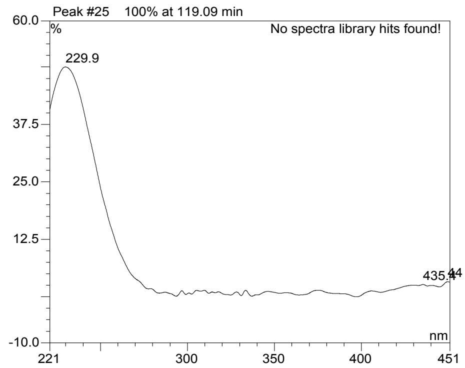
94.03 -

NI 108





95.06 - **NI 109** 97.63 - **NI 110**		
99.70 - **NI 111** 100.96 - **NI 112**		
106.75 - **NI 113** 108.54 - **NI 114**		
111.73 - **NI 115** 115.43 - **NI 116**		

118.53 - **NI 117** 119.09 - **NI 118**		

Zusammenfassung

Mit dieser Masterarbeit wurde versucht verschiedene Fragestellungen zu bearbeiten, die alle im Zusammenhang mit der Dynamik von Wurzelexsudaten stehen.

Zuerst sollte mit einem breit angelegten Versuch ("Artspezifische Variation"/Experiment 1) verifiziert werden, ob die von uns gewählte Methode der Exsudatgewinnung und -aufbereitung sensitiv genug ist, um stabile und reproduzierbare Ergebnisse zu liefern, die auch in Übereinstimmung mit der Literatur stehen.

Weiters sollte die Rolle von Wurzelexsudaten und von Mykorrhizapilzen bei der Mobilisierung von Nährstoffen, im speziellen Phosphor, abgeklärt werden ("Einfluss von Mykorrhizierung"/ Experiment 2), und auch wie sich die Zusammensetzung der Wurzelausscheidungen durch Mykorrhizierung oder Phosphormangel verändert. Ob sich auch im jahreszeitlichen Verlauf Unterschiede zeigen, v.a. hinsichtlich der Benzoesäuregehalte, das war schließlich die dritte Fragestellung ("Saisonale Variation"/Experiment 3).

Für die ersten beiden Experimente wurden verschiedene Nutzpflanzen herangezogen (Tomate, Kartoffel, Gerste und Karotte für beide; Paprika, Gurke, Bohne, Erbse und Weißer Senf nur für das erste), für das letzte einfacherweise 3 Arten aus den gärtnerischen Anlagen.

Die gewonnen Exsudate wurden über Amberlit XAD-1180 aufgetrennt, um eine Wasser- und eine Ethanolphase zu erhalten, die in weiterer Folge mittels GC-MS und HPLC/DAD analysiert wurden - für den Jahreszeitenvergleich wurden nur HPLC-Messungen, da das Hauptaugenmerk auf Unterschieden im relativen Gehalt von Benzoesäure lag, vorgenommen. Die detektierten Metaboliten wurden mittels GC-MS Chromatogrammen und UV/VIS Spektren und dem Vergleich mit Spektrenbibliotheken klassifiziert. Sowohl die GC-MS, als auch die HPLC-Profile wurden weiters mittels Hauptkomponentanalyse (PCA) verglichen.

Das Experiment "Artspezifische Variation" zeigt sowohl hinsichtlich HPLC-, als auch GC-MS Profilen eine deutliche Gruppierung von mehr oder weniger allen Familien (wohingegen die Unterschiede von Arten aus ein- und derselben Familie geringer ausgeprägt waren), mit Ausnahme der *Cucurbitaceae* und *Solanaceae*. Die Variation war dabei aufgrund vieler Gemeinsamkeiten bezüglich der Hauptmetaboliten eher gering, trotzdem war es möglich einige charakteristische Verbindungen zu detektieren.

Der "Einfluss von Mykorrhizierung" zeigte sowohl bezüglich der GC-MS, als auch der HPLC-Profile, nur bei Karotte eine eindeutige Gruppierung der verschiedenen Behandlungen. Eine Gruppe für die mykorrhizierten Pflanzen, und eine für die Kontrollen und diejenigen, die eine höhere P-Düngung erhielten. Das könnte v.a. auf die sehr erfolgreiche Besiedlung (71%) der Karottenwurzeln durch den pilzlichen Partner (*Glomus mossae*) zurückzuführen sein. Die mykorrhizierten Pflanzen zeigten eine weitaus höhere Anzahl (über 50% mehr) an detektierten Verbindungen, sowohl bei den GC-MS, als auch den HPLC-Messungen. Die Unterschiede zwischen den mykorrhizierten Pflanzen und den beiden anderen Behandlungen zeigten sich v.a. bei den Zuckern und Zuckeralkoholen. Interessanterweise, und im Gegensatz zu bisherigen Untersuchungen, exsudierten die mit weniger P-versorgten Kontrollpflanzen nicht mehr und auch keine höheren Anteile an phenolischen Metaboliten. Das kann aber auch daran liegen, dass die Unterversorgung noch nicht ausreichend genug ausgeprägt war.

Der Vergleich der Benzoesäuregehalte in den Wurzelausscheidungen von 3 verschiedenen Arten im Sommer und Winter, zeigte statistisch signifikante Unterschiede. Aber während die beiden krautigen Arten, *Digitalis purpurea* und *Begonia sutherlandii*, höhere Gehalte im Winter zeigten, zeigte die holzige, *Taxus baccata*, geringere. Deshalb kann der höhere Gehalt an Benzoesäure im Winter zwar als Einzelbeobachtung, aber nicht durch eine systematische Untersuchung bestätigt werden. Außerdem wäre es interessant durch weitere Untersuchen zu überprüfen, ob diese Beobachtungen auch bei anderen krautigen und holzigen Arten zutreffen.

Generell führte die PCA der HPLC-Profile zu einer deutlichen Gruppierung zwischen den Jahreszeiten, welche sogar stärker ausgeprägt war, als wie zwischen den einzelnen Arten. Dies traf v.a. für den Sommer zu. Das war weiters darauf begründet, dass im Winter weitaus mehr Metaboliten in den Exsudaten von allen 3 Arten detektiert werden konnten.

Damit kann gesagt werden, dass die Zusammensetzung von Wurzelexsudaten bis zu einem gewissen Punkt auch die phylogenetischen Beziehungen widerspiegelt, aber die Wachstums- und Umweltbedingungen, genauso wie biotische Interaktionen, einen großen Einfluss haben.

Die in dieser Arbeit beschriebene und angewandte Methode der Exsudatgewinnung und -aufbereitung, ist als praktische Anwendung dazu geeignet, qualitative und quantitative Informationen über die Zusammensetzung der Wurzelausscheidungen zu gewinnen. Aber natürlich ist sie nicht perfekt, wie auch keine andere, und hat ihre Limitierungen.

Abschließend bleibt festzuhalten, dass alle der erhaltenen Ergebnisse in größerem Umfang, mit mehr Arten und auch Methoden, noch weiter verifiziert werden müssen, um stichhaltige Aussagen über die untersuchten Phänomene zuzulassen.

Abstract

The aim of this master thesis was to answer several questions related with the dynamic of root exudates.
Firstly it should be verified with a broad experiment, if our method of choice for the collection and processing of root exudates is applicable, in terms of stable and reproducible results, which further are in concordance with literature ("Species Variation"/Experiment 1).
Further on the role of exudates and mycorrhizal fungi for the mobilisation of nutrients, especially phosphorous, should be clarified ("Effect of Mycorrhization"/Experiment 2), and also to which extent the composition of root-exudation is altered through mycorrhization and P-deficiency. If there are also seasonal variations, especially in terms of benzoic acid contents, was the background for the third experiment ("Seasonal Variation").
For the first two experiments several crop plants were used (tomato, potato, barley, and carrot for both; pepper, cucumber, bean, pea and white mustard only for the first one), for the third one just three species from the gardeners facilities due to practical reasons.
The obtained exudates were separated in a hydrophilic- and a lipophilic fraction through Amberlit XAD-1180, and further on analysed with GC-MS and HPLC-UV - for the seasonal variation the analysis was restricted to HPLC-UV, because benzoic acid concentrations were the main focus. Detected metabolites were classified by GC-MS chromatograms and UV/VIS spectra and tentatively identified by an spectra library. GC-MS, as well as HPLC profiles, were analysed by principle component analysis (PCA).
Experiment "Species Variation" showed separate clusters for more or less all families, besides *Cucurbitaceae* and *Solanaceae,* according to GC-MS, as well as to HPLC profiles. Variation itself was rather low, due to several similarities concerning the main components. But still it was possible to detect some characteristic metabolites.
Results of the experiment "Effects of Mycorrhization" showed only separate clusters for the different treatments within carrot, one for the +M-treatment, and one for the control-plants and the +P-treatment. This possibly could be allocated to the very successful colonisation of the roots by the mycorrhizal fungus (71% / *Glomus mossae*). The mycorrhized plants showed a much higher total number of exuded compounds, as well as with GC-MS measurements as with HPLC measurements. The differences between the treatments were mainly founded on sugars and sugar alcohols. Interestingly, and in contradiction to previously published literature, lower phosphorus supplied plants did not exude more phenolic compounds than better supplied. Perhaps the lower supply was not deficient enough.
Comparison of benzoic acid contents throughout the root exudates of three different species in

summer and winter showed statistically significant differences. But as in the woody *Taxus baccata,* benzoic acid concentrations were lower in winter than in summer, in the two herbaceous species, *Digitalis purpurea* and *Begonia sutherlandii,* benzoic acid concentrations were higher in winter than in summer as originally expected. Therefore it can be said that higher contents of benzoic acid can be observed in some species, but it cannot be stated as a general rule. Furthermore it would be interesting if investigations including more accessions of herbaceous and woody plants will show if this phenomenon represents a robust characteristic. Generally PCA resulted in separate clusters for the two seasons. The variation between the two seasons was much stronger than between the three species, especially during summer. In winter there could be much more metabolites detected in the exudates of all three species.
Therefore it can be said, that the composition of root exudates is reflecting phylogenetic relationships to some extent, but also cultivation and environmental conditions, as well as biotic interactions are having a great influence.
Our method of choice for the collection and processing of root exudates, is a practical approach to get qualitative and quantitative information about the composition of root exudates. But for sure it's not perfect, like no other, and has its limitations.
Concluding it has to be declared, that all of the obtained results have to be verified in a larger scale, with more species and accessions, to allow substantive propositions concerning the examined phenomenons.

Keywords:
Root exudates, exudate dynamics, exudate diversity, mycorrhization, phosphorous nutrition, benzoic acid, seasonal variation, crop plants, GC-MS, HPLC-UV, PCA.

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Curriculum vitae

Personal Information

Surname(s) / First Name(s)	**Peter, Jeremy-James**
Address(es)	Machstrasse 3/2/8, 1020 Wien, Vienna, Austria
Nationality	Austria
Date of Birth	1980-31-12

Education

January 2010	BSc in biology, inscription in master study plant sciences
October 2007	Master thesis at department: Department of Chemical Ecology and Ecosystem Research, University of Vienna, master subject: Phytochemistry and physiology
November 2004	First diploma degree in biology, University of Vienna and inscription in second study phase of biology, branch of study: Botany (A 438), University of Vienna.
October 2001	Inscription in biology (A437), University of Vienna
June 2000	Graduation at BORG Feldkirch, grammar school with focus on natural sciences.